GE Healthcare

Getting Started with

ÄKTAavant™ and UNICORN™ 6

ÄKTA™

Table of Contents

1 About this guide

Introduction

This chapter describes the purpose of the guide and provides regulatory information relevant for ÄKTAavant system.

Contents

This chapter contains the following sections:

1.1 Get started

Introduction

Read this section for an understanding of the purpose and conventions of this guide, as well as the requirements that you must fulfill before using the ÄKTAavant system.

Purpose of Getting Started

The purpose of this guide is to present a quick and easy guide to the system for a user with limited or no experience of UNICORN software and ÄKTAavant instrument. The work flow is presented as practical instructions on how to operate the software and the instrument. The instructions form a basic framework that you can expand on by reading selected parts in the other manuals. This Getting Started guide includes the following topics:

- Basic features of ÄKTAavant and UNICORN
- Create methods
- Prepare the system for runs
- Perform runs
- Make simple evaluations
- Print reports

For best results, follow the guide from page to page in front of the system.

Prerequisites

In order to follow this guide and use the system in the manner it is intended, it is important that:

- you have a general understanding of how the computer and Windows™ work.
- you understand the concepts of liquid chromatography.
- you have read and understood the Safety instructions chapter in the user documentation.
- the instrument and software are installed, configured and calibrated according to *ÄKTAavant and UNICORN 6 Installation Guide*.
- a user account has been created according to *UNICORN 6 Administration and Technical Manual*.

Pushing the limits beyond Getting Started

Users who are familiar with the instrument and software and want to learn more about the system's advanced features should refer to the list below.

To find out more about...	please read..
installation	*ÄKTAavant and UNICORN 6 Installation Guide*
administration of databases	*UNICORN 6 Administration and Technical Manual*
instrument modules and functions	*ÄKTAavant and UNICORN 6 User Manual*
calibration	*ÄKTAavant and UNICORN 6 User Manual*
predefined methods	*UNICORN 6 Method Manual*
column handling	*UNICORN 6 Method Manual*
manual method editing	*UNICORN 6 Method Manual*
BufferPro–automatic buffer preparation	*UNICORN 6 Method Manual*
scouting	*UNICORN 6 Method Manual*
Design of Experiment	*UNICORN 6 Method Manual*
evaluation	*UNICORN 6 Evaluation Manual*
peak integration	*UNICORN 6 Evaluation Manual*
customized print format	*UNICORN 6 Evaluation Manual*

Intended use

ÄKTAavant is a liquid chromatography system intended for method development. The system can be used to screen for optimal choice of columns, media and running parameters to purify selected proteins.

The ÄKTAavant system is intended for research use only, and shall not be used in any clinical procedures, or for diagnostic purposes.

Safety notices

This user documentation contains WARNINGS, CAUTIONS and NOTICES concerning the safe use of the product. See definitions below.

Warnings

WARNING

WARNING indicates a hazardous situation which, if not avoided, could result in death or serious injury. It is important not to proceed until all stated conditions are met and clearly understood.

Cautions

CAUTION

CAUTION indicates a hazardous situation which, if not avoided, could result in minor or moderate injury. It is important not to proceed until all stated conditions are met and clearly understood.

Notices

NOTICE

NOTICE indicates instructions that must be followed to avoid damage to the product or other equipment.

Typographical conventions

Software items are identified in the text by ***bold italic*** text. A colon separates menu levels, thus ***File:Open*** refers to the ***Open*** command in the ***File*** menu.

Hardware items are identified in the text by **bold** text (e.g., **Power** switch).

Text entries that UNICORN generates or that the user must type are represented by a monotype typeface (e.g., `\Program Files\GE Healthcare\UNICORN\bin\UNICORN Instrument Server.exe.config`).

1.2 Regulatory information

Introduction

This section describes the directives and standards that are fulfilled by the ÄKTAavant system.

CE conformity

This product complies with the European directives listed in the table below, by fulfilling the corresponding harmonized standards. A copy of the Declaration of Conformity is available on request.

Directive	Title
2006/42/EC	Machinery Directive
2006/95/EC	Low Voltage Directive
2004/108/EC	ElectroMagnetic Compatibility (EMC) Directive
1999/5/EC	Radio Equipment and Telecommunications Terminal Equipment Directive

CE marking

CE

The **CE** marking and the corresponding declaration of conformity, is valid for the instrument when it is:

- used as a stand-alone unit, or
- connected to other **CE** marked GE Healthcare instruments, or
- connected to other products recommended or described in the user documentation, and
- used in the same state as it was delivered from GE Healthcare, except for alterations described in the user documentation.

International standards

This product fulfills the requirements of the following standards:

Standard	Description	Notes
EN 61010-1, IEC 61010-1, UL 61010-1, CAN/CSA-C22.2 No.61010-1	Safety requirements for electrical equipment for measurement, control, and laboratory use	
EN 61326-1	EMC emissions and immunity requirements for electrical equipment for measurement, control and laboratory use.	Harmonized with 2004/108/EC CISPR 11 Group 1, Class B
EN-ISO 12100-1, 12100-2	Safety of machinery — Basic concepts, general principles for design	Harmonized with 2006/42/EC
EN ISO 14121-1, 14121-2	Safety of machinery — Principles of risk assessment	Harmonized with 2006/42/EC

Software declaration of conformity

UNICORN 6 is technically compatible with all relevant sections of FDA 21 CFR Part 11.

A part 11-system assessment checklist is available on request through the local GEHC representative.

Regulatory compliance of connected equipment

Any equipment connected to the ÄKTAavant system should meet the safety requirements of EN 61010-1/IEC 61010-1, or other relevant harmonized standards. Within EU, connected equipment must be CE marked.

2 Safety instructions

Introduction

This chapter contains instructions of how to handle the ÄKTAavant instrument in a safe way.

Contents

This chapter contains the following sections:

2.1 Safety precautions

Introduction

The ÄKTAavant instrument is powered by mains voltage and handles pressurized liquids that may be hazardous. Before installing, operating or maintaining the system, you must be aware of the hazards described in this manual. ***Follow the instructions provided to avoid personal injuries or damage to the equipment***.

The safety precautions in this section are grouped into the following categories:

- General precautions
- Personal protection
- Installing and moving the instrument
- System operation
- Maintenance

General precautions

WARNING

Always follow these General precautions to avoid injury when using the ÄKTAavant instrument.

- Do not operate the ÄKTAavant instrument in any other way than described in the ÄKTAavant and UNICORN manuals.
- Operation and user maintenance of the ÄKTAavant instrument should be performed by properly trained personnel only.
- Before connecting a column to the ÄKTAavant instrument, read the instructions for use of the column. To avoid exposing the column to excessive pressure, make sure that the pressure limit is set to the specified maximum pressure of the column.
- Do not use any accessories not supplied or recommended by GE Healthcare.
- Do not use the ÄKTAavant instrument if it is not working properly, or if it has suffered any damage, for example:
  - damage to the power cord or its plug
  - damage caused by dropping the equipment
  - damage caused by splashing liquid onto it

NOTICE

Avoid condensation by letting the instrument equilibrate to ambient temperature.

Using flammable liquids

WARNING

When using flammable liquids with the ÄKTAavant instrument, follow these precautions to avoid any risk of fire or explosion.

- **Fire Hazard**. Before starting the system, make sure that there are no unintentional leakage in the instrument or tubing.
- **Explosion hazard**. To avoid building up an explosive atmosphere when using flammable liquids, make sure that the room ventilation meets the requirements specified in *ÄKTAavant and UNICORN 6 Installation Guide*.
- **Fraction collector**. Do *not* fractionate flammable liquids. When running RPC methods, or other procedures using solvent based buffers, collect fractions through the outlet valve.

Personal protection

Warnings

WARNING

To avoid hazardous situations when working with the ÄKTAavant system, take the following measures for personal protection.

- Always use appropriate personal protective equipment during operation and maintenance of ÄKTAavant system.
- **Hazardous substances.** When using hazardous chemical and biological agents, take all suitable protective measures, such as wearing protective glasses and gloves resistant to the substances used. Follow local and/or national regulations for safe operation and maintenance of the system.

- **Spread of biological agents**. The operator has to take all necessary actions to avoid spreading hazardous biological agents in the vicinity of the instrument. The facility should comply with the national code of practice for biosafety.
- **High pressure.** The ÄKTAavant instrument operates under high pressure. Wear protective glasses at all times.

Cautions

CAUTION

To avoid hazardous situations when working with the ÄKTAavant system, take the following measures for personal protection.

- **Close doors.** To minimize the risk of exposure to hazardous chemicals and pressurized liquids, always close the Foldable door and the Pump cover before starting a run.
- **Cut injuries.** The tubing cutter is very sharp and must be handled with care to avoid injuries.

Installing and moving the instrument

Warnings

WARNING

To avoid personal injury when installing or moving the ÄKTAavant instrument, follow the instructions below.

- **Heavy object.** The ÄKTAavant instrument weighs about 100 kg. Use proper lifting equipment, or use four or more persons when moving the instrument. All lifting and moving must be performed in accordance with local regulations.
- **Moving the instrument horizontally**. Three persons are recommended when moving the instrument horizontally.
- **Supply voltage.** Make sure that the supply voltage at the wall outlet corresponds to the marking on the instrument, before connecting the power cord.
- **Protective ground.** The ÄKTAavant instrument must always be connected to a grounded power outlet.
- **Power cord**. Only use power cords delivered or approved by GE Healthcare.

- **Access to power switch and power cord**. Do not block the rear and side panel of the instrument. The **Power** switch must always be easy to access. The power cord must always be easy to disconnect.
- **Installing the computer**. The computer should be installed and used according to the instructions provided by the manufacturer of the computer.

Notices

NOTICE

To avoid damage to ÄKTAavant or other equipment when installing or moving the instrument, follow the instructions below.

- **Vents on the ÄKTAavant instrument**. To ensure adequate ventilation, keep papers and other objects away from the vents of the instrument.
- **Disconnect power.** To prevent equipment damage, always switch off power to the ÄKTAavant instrument before an instrument module is removed or installed, or a cable is connected or disconnected.
- **Misuse of UniNet-9 connectors.** The **UniNet-9** connectors at the rear panel should not be mistaken for Firewire connectors. Do not connect any external equipment to the **UniNet-9** connectors. Do not disconnect or move the **UniNet-9** bus cable.

System operation

Warnings

WARNING

To avoid personal injury when operating the ÄKTAavant system, follow the instructions below.

- **Rotating the instrument.** Make sure that there is always at least 20 cm of free space around the ÄKTAavant instrument to allow rotation on the swivel foot. When rotating the system, take care not to stretch or squeeze tubing or cables. A disconnected cable may cause power interruption or network interruption. Stretched tubes may cause bottles to fall, resulting in liquid spillage and shattered glass. Squeezed tubing may cause increase in pressure, or block liquid flow. To avoid the risk of turning over bottles, always place bottles on the buffer tray, and close the doors before rotating the instrument.

- **Fasten sample bottles**. Always fasten bottles and cassettes to the rails at the sample tray. Use appropriate holders for bottles. Shattered glass from falling bottles may cause injury. Spilled liquid may cause fire hazard and personal injury.

- **Electrical shock hazard after spillage.** If there is a risk that large volumes of spilled liquid may penetrate the casing of the ÄKTAavant instrument, immediately switch off the instrument, disconnect the power cord, and contact an authorized service engineer.
- **Moving parts in Fraction collector**. Do not open the Fraction collector drawer when the Fraction collector is active. If you need to access the Fraction collector, press ***Pause***, and make sure that the movement has stopped before opening the drawer.
- **Using a Superloop**. After loading a Superloop, always plug the **Syr** port on the Injection valve with a Stop plug. With a Superloop connected to the valve, an over-pressure may be created during injection.
- **Hazardous chemicals during run.** When using hazardous chemicals, run ***System CIP*** and ***Column CIP*** to flush the entire system tubing with distilled water, before service and maintenance.
- **Hazardous biological agents during run.** When using hazardous biological agents, run ***System CIP*** and ***Column CIP*** to flush the entire system tubing with bacteriostatic solution (e.g., NaOH) followed by a neutral buffer and finally distilled water, before service and maintenance.
- **RPC runs with 100% acetonitrile**. Always replace the green PEEK tubing between the used system pump and the pressure monitor with orange PEEK tubing, i.d. 0.5 mm, before running RPC with 100% acetonitrile. Set the System pressure alarm to 10 MPa.

Cautions

CAUTION

To avoid hazardous situations when operating the ÄKTAavant system, follow the instructions below.

- **Risk of breaking test vials**. Do not use excessive force to press vials with incorrect dimensions into the Fraction collector cassettes. Glass vials may break and cause injuries.
- **Hazardous chemicals or biological agents in UV flow cell**. Make sure that the entire flow cell has been flushed thoroughly with bacteriostatic solution (e.g., NaOH) and distilled water, before service and maintenance.
- **pH-electrode**. Handle the pH-electrode with care. The glass tip may break and cause injury.

- **Max. weight on top tray**. Do not place containers with a volume of more than 10 liters on the top tray. The total allowed weight on the top tray is 40 kg.
- **Max. size of bottles on front panel**. Do not fasten bottles with a volume of more than 1 liter in the front panel rails.

Notices

NOTICE

To avoid damage to ÄKTAavant or other equipment when operating the instrument, follow the instructions below.

- **Keep UV flow cell clean.** Do not allow solutions containing dissolved salts, proteins or other solid solutes to dry out in the flow cell. Do not allow particles to enter the flow cell, as damage to the flow cell may occur.
- **Waste container height**. The maximum level of the waste container content must be lower than 30 cm above the lab bench, for trouble-free use.

Maintenance

Warnings

WARNING

To avoid personal injury when performing maintenance on the ÄKTAavant instrument, follow the instructions below.

- **Electrical shock hazard.** All repairs should be done by service personnel authorized by GE Healthcare. Do not open any covers or replace parts unless specifically stated in the user documentation.
- **Disconnect power.** Always switch off power to the instrument before replacing any component on the instrument, unless stated otherwise in the user documentation.
- **Hazardous chemicals during maintenance.** When using hazardous chemicals for system or column cleaning, wash the system or columns with a neutral solution in the last phase or step.

NOTICE

Cleaning. Keep the instrument dry and clean. Wipe regularly with a soft damp tissue and, if necessary, a mild cleaning agent. Let the instrument dry completely before use.

2.2 Labels

Introduction

This section describes the safety labels and labels concerning hazardous substances that are attached to the ÄKTAavant instrument. For information about marking of the computer equipment, refer to the manufacturer's instructions.

Labels on the ÄKTAavant instrument

The illustrations below show the labels that are attached to the ÄKTAavant instrument.

Safety symbols

The following safety symbols are used in the labels:

Label	Meaning
	**Warning!** **Electrical shock hazard.** All repairs should be done by service personnel authorized by GE Healthcare. Do not open any covers or replace parts unless specifically stated in the user documentation. **Supply voltage.** Make sure that the supply voltage at the wall outlet corresponds to the marking on the instrument, before connecting the power cord.
	**Warning!** **Fraction collector**. Do *not* fractionate flammable liquids. When running RPC methods, or other procedures using solvent based buffers, collect fractions through the outlet valve.
	The system complies with the requirements for electromagnetic compliance (EMC) in Australia and New Zealand.
	The system complies with applicable European directives.

Labels concerning hazardous substances

The following symbols on the labels concern hazardous substances:

Label	Meaning
	This symbol indicates that electrical and electronic equipment must not be disposed of as unsorted municipal waste and must be collected separately. Please contact an authorized representative of the manufacturer for information concerning the decommissioning of equipment.
	This symbol indicates that the product contains hazardous materials in excess of the limits established by the Chinese standard *SJ/T11363-2006 Requirements for Concentration Limits for Certain Hazardous Substances in Electronic Information Product*.

2.3 Emergency procedures

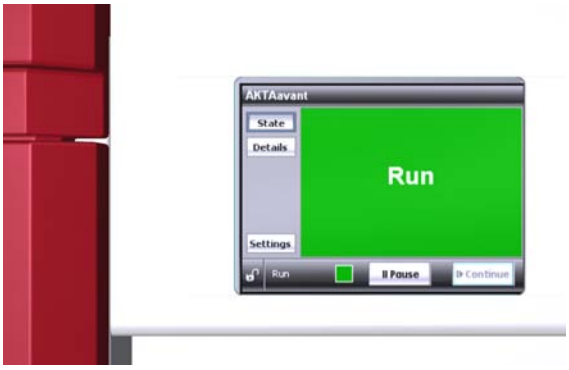
Introduction

This section describes how to do an emergency shutdown of the ÄKTAavant system. The section also describes the result in the event of power failure or network interruption.

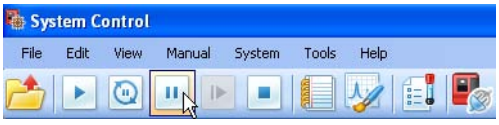
Emergency shutdown

In an emergency situation, follow the steps below to stop the run:

Step	Action
1	Press the **Pause** button on the instrument display. This will stop all pumps in the instrument.



To pause the run from UNICORN, click the ***Pause*** icon:



Step	Action
2	If required, switch off power to the instrument by pressing the **Power** switch to the **O** position. The run is interrupted immediately.

Power failure

The result of a power failure depends on which unit is affected.

Power failure to...	will result in...
ÄKTAavant instrument 	• The run is interrupted immediately • The data collected up to the time of the power failure is available in UNICORN.

Power failure to...	will result in...
Computer 	• The UNICORN computer shuts down • The instrument display shows status ***Not connected*** • The run is interrupted immediately • Data generated up to 10 seconds before the power failure can be recovered ***Note:*** *The UNICORN client may close down during a temporary overload of the processor. This may appear as a computer failure. The run continues and you can restart the UNICORN client to regain control.*

Uninterruptible power supply (UPS)

A UPS can eliminate data loss after a power failure, and allow time for a controlled shut-down of the ÄKTAavant system.

3 Start up

Introduction

This chapter describes how to start the system, both the software and the instrument, and also how to log off and exit UNICORN.

It is assumed that your user profile is already created. For information about how to set up and define users, user groups and access rights, please refer to *UNICORN 6 Administration and Technical Manual*.

Contents

This chapter contains the following sections:

3.1 Start the system and log on to UNICORN

Introduction

This section describes how to start UNICORN and the instrument, and how to connect to the system.

Start UNICORN and log on

Follow the instruction below to start and log on to UNICORN.

Step	Action
1	To start the program, double-click the UNICORN icon on the desktop.

Result: The ***Log On*** dialog opens.

Step	Action
2	In the ***Log On*** dialog: • Select your ***User Name*** from the drop-down list. • Enter your ***Password***. • Click the ***Options*** button.  *Result*: Checkboxes for each of the UNICORN modules are displayed in the ***Log On*** dialog. 
3	In the ***Log On*** dialog: • Select which UNICORN modules to start by clicking the corresponding checkboxes. • Click ***OK***. *Result*: The selected UNICORN modules open.

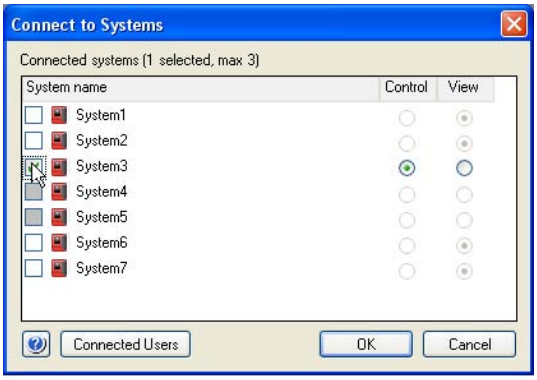
Start the instrument and connect to the system

Follow the instruction below to start the instrument and connect to the UNICORN software.

Step	Action
1	Switch on the instrument by pressing the **Power** switch to the **I** position.

2	Open the the ***System Control*** module in UNICORN and click the ***Connect to Systems*** icon.

Result: The ***Connect to Systems*** dialog opens.



Step	Action
3	In the ***Connect to Systems*** dialog: • Select the instrument. • Select ***Control*** mode. • Click ***OK***. *Result:* The selected instrument can now be controlled by the software.

4 The instrument and the software

Introduction

This chapter gives an overview of the ÄKTAavant system: instrument, software and accessories.

Illustration of the system

The illustration below shows the ÄKTAavant instrument with UNICORN software installed on a computer.

Contents

This chapter contains the following sections:

4.1 ÄKTAavant instrument overview

Introduction

This section provides an overview of the ÄKTAavant instrument. Technical details about the instrument and the individual modules are found in *ÄKTAavant and UNICORN 6 User Manual*.

Exterior design

ÄKTAavant has a modular design, with all the liquid handling modules placed on the exterior of the instrument. Buffer vessels are placed on the Buffer tray on top of the instrument. An Instrument display is placed on the front. From this side the built-in Fraction collector is handled, as well as the sample. The rest of the modules are placed on the right-hand side of the instrument. This side can be covered by a foldable door and a pump cover. By rotating the instrument using the swivel foot any side is easily accessed.

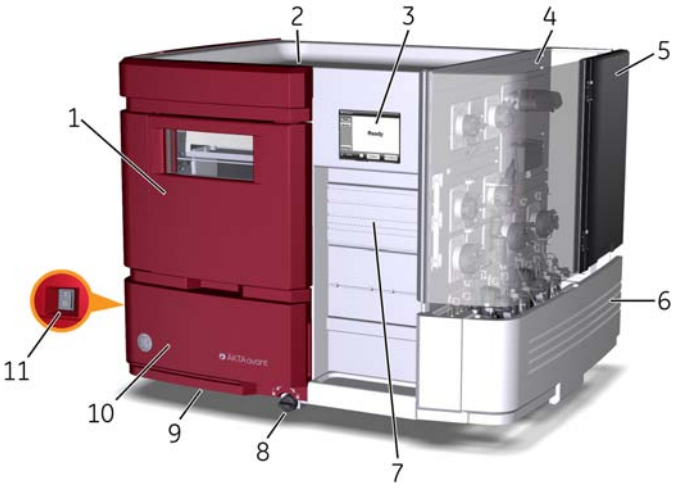
Operating ranges

The table below shows some of the instrument's operational limits.

Parameter	Limits
Flow rate	Up to 25 ml/min
Max. operating pressure	20 MPa
Wavelength	190 - 700 nm

Illustration of the main parts of the instrument

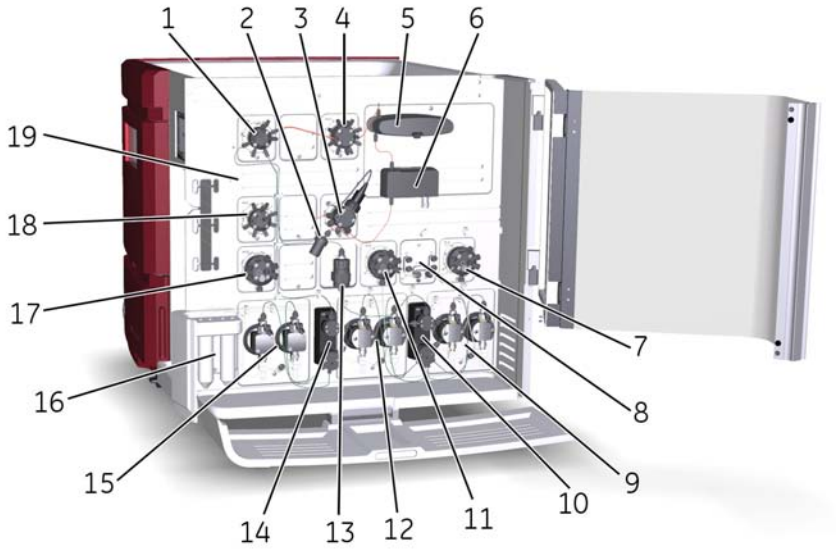
The illustration below shows the location of the main parts of the instrument.



Part	Description
1	Fraction collector
2	Buffer tray
3	Instrument display
4	Wet side
5	Foldable door
6	Pump cover
7	Holder rails
8	Swivel foot **Lock/Unlock** knob
9	Swivel foot
10	Swing out toolbox
11	**Power** switch

Illustration of the wet side modules of the instrument

The illustration below shows the modules of the wet side of the instrument.



Part	Description
1	Injection valve
2	Column valve
3	Flow restrictor
4	pH valve
5	UV monitor
6	Conductivity monitor
7	Inlet valve B
8	Quaternary valve
9	System pump B
10	Pressure monitor of System pumps
11	Inlet valve A

Part	Description
12	System pump A
13	Mixer
14	Pressure monitor of Sample pump
15	Sample pump
16	Pump piston rinsing system tubes
17	Sample inlet valve
18	Outlet valve
19	Holder rails

4.2 UNICORN software overview

Introduction

This section gives a brief overview of the UNICORN software—a complete package for control, supervision and evaluation of chromatography instruments and purification runs. It also describes how to access the help utility that is included in UNICORN.

UNICORN modules overview

UNICORN consists of four modules: ***Administration***, ***Method Editor***, ***System Control*** and ***Evaluation***. The main functions of each module are described in the table below.

Module	Main functions
Administration	Perform user and system setup, system log and database administration.
Method Editor	Create and edit methods.
System Control	Start, view and control runs.
Evaluation	Open results, evaluate runs and create reports.

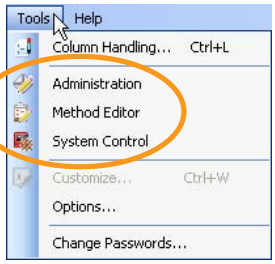
Enter a UNICORN module

To enter a module:

- click the ***Taskbar*** button of the module of interest,

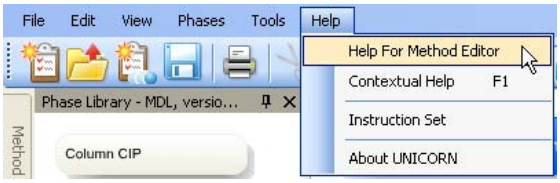
or

- choose the module of interest in the ***Tools*** menu in any of the other software modules. The illustration below shows the ***Tools*** menu of the ***Evaluation*** module.



Access the help utility

A comprehensive help utility is included in the UNICORN software. The table below describes how to access the different parts of the help utility.

If you want to...	then...
find information about a UNICORN module	select ***Help:Help for...*** in the UNICORN module of interest 
find information about the item currently selected and in focus (e.g., a pane, a dialog, or a method phase)	• press the **F1** key with the item of interest selected and in focus ***Note:*** *To find information about a phase, make sure that the **Phase Properties** tab is selected before pressing the **F1** key.* or • click the ***Help*** icon in the open dialog 

If you want to...	then...
navigate the online help	• select ***Help:Help for...*** in any of the UNICORN modules (see illustration above) • in the ***TOC*** pane, expand the headings of interest to navigate the content structure • click the heading of interest to open a section
search for a specific term in the online help	• select ***Help:Help for...*** in any of the UNICORN modules (see illustration above) • in the ***Search*** pane, enter the term of interest in the input field • click the ***Search*** button 
access any of the manuals in PDF format	• select ***Help:Help for...*** in any of the UNICORN modules (see illustration above) • in the ***TOC*** pane, expand the heading ***UNICORN online documentation portal*** and select ***Online documentation overview*** • in the ***PDF manuals*** section, click one of the text links • click the illustration or the text link of the manual of interest
find information about a method instruction	In the ***Method Editor*** module: • open a method • select the instruction of interest in the ***Instruction box*** in the ***Text instruction*** pane • press the **F1** key In the ***System Control*** module: • select ***Manual:Execute Manual Instructions*** • expand a heading and select the instruction of interest • click the ***Help*** icon in the dialog 

4.3 UNICORN software modules

Introduction

Three of the four UNICORN modules are used in this Getting Started guide and described in this section: ***Method Editor***, ***System Control*** and ***Evaluation***. The ***Administration*** module and its icons are described in *UNICORN 6 Administration and Technical Manual*.

Contents

This section contains the following subsections:

4.3.1 Method Editor module

Introduction

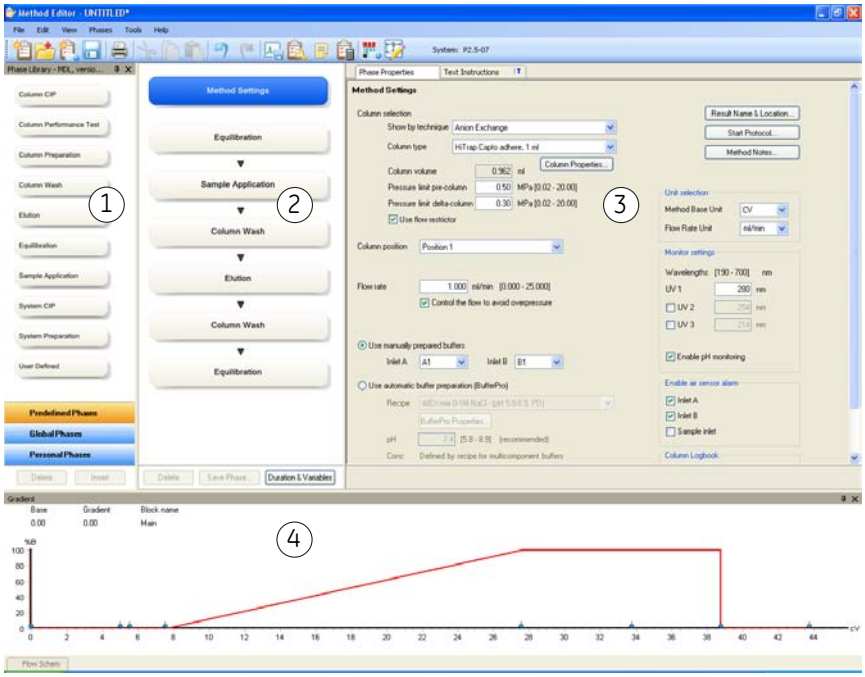
In UNICORN software, the instructions to control a chromatography run are defined in a method. The ***Method Editor*** module is used to create or edit such methods. The graphical user interface combines default settings with the ability to change settings. This simplifies method creation and replaces the method wizard in earlier versions of UNICORN.

Predefined methods and phases

A method is built up by a number of phases. Each phase represents a major process step in the method, for example, equilibration or elution. Predefined methods, that include all the phases necessary to run the system, are available for different chromatography techniques and also for system cleaning.

Method Editor panes

As illustrated below, four panes show in the ***Method Editor*** by default. Available phases are found in the ***Phase Library*** (1), and an overview of the phases included in the active method is displayed in a ***Method Outline*** (2). Detailed information about the method is presented in the upper right-hand pane (3), containing the two tabs ***Phase Properties*** and ***Text Instructions***. The gradient used in the method is displayed in a ***Gradient*** illustration (4).



Method Editor toolbar icons

The table below shows the ***Method Editor*** toolbar icons that are referred to in this Getting Started guide.

Icon	Function	Icon	Function
	***New Method:*** Opens the ***New Method*** dialog where methods can be created.		***Open Method Navigator***: Opens the ***Method Navigator*** where available methods are listed.
	***Save***: Saves the active method.		***Print***: Opens the ***Print*** dialog from where a method can be printed.
	***Copy***: Copies the selected method or folder.		***Paste***: Pastes a copied method or folder to a new location.
	***Undo***: Restores the method to the state it was in before the last change.		***Redo***: Restores the method to the state it was in before the ***Undo*** command was used.

4.3.2 System Control module

Introduction

The ***System Control*** module is used to start, view, and control a method run.

System Control panes

As illustrated below, four panes show in the ***System Control*** by default. The ***Run Data*** pane (1) presents current data in numerical values, while the ***Chromatogram*** pane (2) illustrates data as curves during the entire method run. Information about the method progression is presented in the ***Run Log*** (3) and the current flow path is illustrated in the ***Flow Scheme*** (4).

System Control toolbar icons

The table below shows the System Control toolbar icons that are referred to in this Getting Started guide.

Icon	Function	Icon	Function
	***Open Method Navigator***: Opens the ***Method Navigator*** where available methods are listed.		***Run***: Starts a method run.
	***Hold***: Suspends the method run, while current flow rate and valve positions are sustained.		***Pause***: Suspends the method run and stops all pumps.
	***Continue***: Resumes for example a held or paused method run.		***End***: Permanently ends the method run.
	***Customize***: Opens the ***Customize*** dialog where curve settings, run data groups and run log contents can be set.		***Connect to Systems***: Opens the ***Connect to Systems*** dialog where systems can be connected, and currently connected users are displayed.

4.3.3 Evaluation module

Introduction

The ***Evaluation*** module is used to evaluate the results from chromatography runs.

Evaluation panes

As illustrated below, the ***Evaluation*** module contains three panes. When a result is opened from the ***Result Navigator*** (1) the ***Chromatogram*** pane (2) is displayed. After a peak integration has been performed, details about the peaks are shown in the lower right-hand pane (3), in the tabs ***Peak data*** and ***Integration summary***. In the ***Evaluation*** module it is also possible to view the complete documentation about the results, and to generate reports.

Evaluation toolbar icons

The table below shows the ***Evaluation*** toolbar icons that are referred to in this Getting Started guide.

Icon	Function	Icon	Function
	***Open Result Navigator***: Opens the ***Result Navigator*** where available results are listed.		***Save***: Saves the changes made to the current result.
	***Print***: Opens the ***Print chromatograms*** dialog from where a chromatogram can be printed.		***Copy***: Copies the selected result or folder.
	***Paste***: Pastes a copied result or folder to the selected folder.		***Undo***: Restores the method to the state it was in before the last change.
	***Report***: Opens the ***Create report*** dialog where a report of the result can be created.		***View Documentation***: Opens the ***Documentation*** dialog that contains the complete documentation for a method run.
	***Customize***: Opens the ***Customize*** dialog where curve settings, peak table content and header content can be set.		***Peak Integrate***: Opens the ***Peak Integrate*** dialog from where curves can be integrated.

5 Files and folders in UNICORN

Introduction

In the UNICORN user interface, method and result data are represented by files. All users have a designated home folder where all methods and result files created by the user will automatically be located. The files and folders are displayed in ***Navigator*** panes.

This chapter briefly describes the ***Navigator*** panes available in the different modules. It also describes how to handle the files and folders.

Contents

This chapter contains the following sections:

5.1 Navigator overview

Introduction

This section describes the navigator panes that are available in all UNICORN modules, with exception for the ***Administration*** module. The navigator panes can be used to locate and open methods and results, and to handle files and folders.

In the modules ***Method Editor*** and ***System Control***, the navigator panes are called ***Method Navigator*** and are used primarily to access methods.

In the ***Evaluation*** module, the navigator pane is called ***Result Navigator***. In this navigator pane three different panes can be used to access the results–***Results***, ***Recent Runs*** and ***Find Results***.

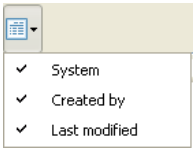
Show the navigator pane

To open and display the navigator pane in any of the UNICORN modules, click the ***Open...*** toolbar icon.

The navigator panes may either be displayed statically in the position where they open, or the ***Auto Hide*** function can be selected by clicking the pin symbol in the top right-hand corner of the pane (illustrated below). If ***Auto Hide*** is selected, the panes open automatically when the mouse pointer is placed over the corresponding tab, and remain open as long as the mouse pointer remains over the pane. The pane closes automatically when the pointer is moved outside.

Navigator toolbar icons

The table below shows the common navigator toolbar icons.

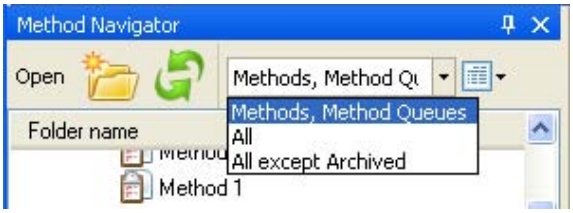
Icon	Function
	***Open***: Opens the selected file.
	***New folder***: Creates a new folder in the folder that is currently selected.
	***Refresh***: Updates all items in the navigator pane to the current status.
	Opens the ***View Details*** droplist (see illustration below) where the following optional information may be selected for display in the navigator pane: • ***System*** • ***Created by*** (user name of the person who created the original file) • ***Last modified*** (date and time when then file or folder was last modified) 

Filter settings

By default, the ***Method Navigator*** shows only methods, and the ***Result Navigator*** only results. This is the ***Default Filter*** setting for the items that are displayed. However, each navigator may also show items that primarily are used in another UNICORN module. The options are:

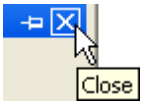
- ***Default Filter***: only items belonging to the active module show
  - In ***Method Editor*** and ***System Control***: "Methods, Method Queues"
  - In ***Evaluation***: "Results, DoE Results"
- ***All***: all items show, regardless of the active module
- ***All except Archived***: only items that are not archived show

The illustration below shows the filter settings available in the ***Method Navigator***.



Close the navigator pane

Click the small cross in the top right-hand corner of the pane to close the navigator pane.



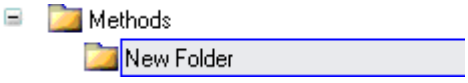
5.2 Handling files and folders

Introduction

The handling of files and folders in UNICORN is similar to the general Windows functions. This section describes how to create new folders and how to copy, move, delete and rename both files and folders. It also describes the general search function that helps you find the files and folders that you are looking for.

Create a folder

To arrange your files for better overview, you can create sub-folders in any folder that you have access to. Follow the instruction below to create a new folder in the ***Method Navigator*** or in the ***Result Navigator***.

Step	Action
1	Select the folder in which you want to create the new sub-folder.
2	Click the ***New Folder*** icon.  *Result*: A sub-folder named "New Folder" is created in the selected folder.  ***Note:*** *The new folder is placed after all other sub-folders, but before any files directly in the selected folder.*
3	Type a name for the new folder. *Result*: The new folder is renamed.

Copy and paste files and folders

Follow the instruction below to copy and paste files and folders in the ***Method Navigator*** or in the ***Result Navigator***. If you copy a folder you will also copy all the files and folders that it contains.

Step	Action
1	Select one or several files and/or folders.
2	Click the ***Copy*** toolbar icon.  *Result:* The selected items are copied.
3	Select a target folder.
4	Click the ***Paste*** toolbar icon.  *Result:* The copied items are pasted into the selected folder.

TIP: You can select a range of items by pressing the keyboard **Shift** key or several individual items by pressing the keyboard **Ctrl** key

Move files and folders

Files and folders may be moved by drag-and-drop within the folder structure that you have access to. The destination must always be a folder and the contents will normally be re-sorted automatically so that:

- folders are placed first, in alphabetical order, and
- individual files are placed in alphabetical order, after the folders.

Note: *The sorting order may be changed by clicking the header of each column. You can also move folders and files by using **Cut** and **Paste** in the same manner as **Copy** and **Paste**.*

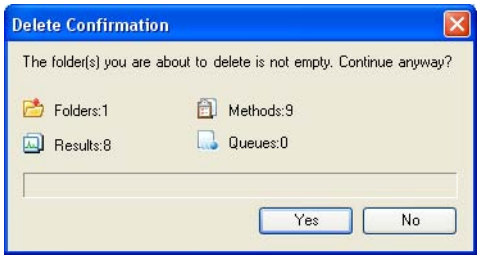
Delete files and folders

Follow the instruction below to delete files and folders in the ***Method Navigator*** or in the ***Result Navigator***.

Note: *Top level folders, for example home folders, cannot be deleted this way. Refer to UNICORN 6 Administration and Technical Manual for more information about home folders.*

Step	Action
1	Select the item(s) that you want to delete.
2	Choose ***Edit:Delete***. *Result:* A dialog for confirmation of the delete action opens.
3	Press ***Yes*** to confirm the delete action in the confirmation dialog. *Result:* The selected items are deleted.

Note: *If you have chosen to delete a folder that is not empty, a special confirmation dialog opens. This dialog displays all the items that will be deleted as a consequence, both items in the active UNICORN module and items in other UNICORN modules (see illustration below).*



Rename files and folders

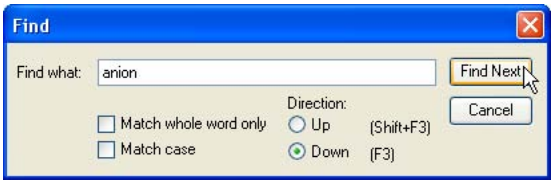
Follow the instruction below to rename files and folders in the ***Method Navigator*** or in the ***Result Navigator***.

Step	Action
1	Select the item that you want to rename.
2	Choose ***Edit:Rename***. *Result*: The current name is selected.
3	Type a new name.

Step	Action
4	Press **Enter**. *Result*: The selected item is renamed.

Find files and folders

Follow the instruction below to use the ***Find*** dialog to search for files or folders in the ***Method Navigator*** or in the ***Result Navigator***.

Step	Action
1	• Select the file or folder in the navigator pane from where you want to perform the search. • Choose ***Edit:Find***. *Result*: The ***Find*** dialog opens. 
2	• Type a name or a partial name in the ***Find what*** field. • Select none, one, or both of the the matching levels described below. - ***Match whole word only***: Matches only whole words and disregards partial matches. - ***Match case***: Matches the case of the ***Find what*** field entry. • Select a ***Direction*** for the search. - ***Up***: The search proceeds upwards in the folder structure from the selected level. - ***Down***: The search proceeds downwards in the folder structure from the selected level.
3	Click ***Find Next***. *Result*: The first located match is selected in the navigator pane. The search can be continued step by step until the whole folder structure has been searched.

6 Create a method

Introduction

A method is created in the ***Method Editor*** module. This chapter describes method creation based on predefined methods, and also how to print a method.

Contents

This chapter contains the following sections:

6.1 Guide to method creation

Introduction

This section describes how to create a new method based on a predefined method, and how to edit a method. The section also provides an overview of the predefined methods and phases that are available. You can create both purification methods and maintenance methods. You can also add a maintenance phase at the end of a purification method. For further information about method creation, see *UNICORN 6 Method Manual*.

Contents

This sections contains the following subsections:

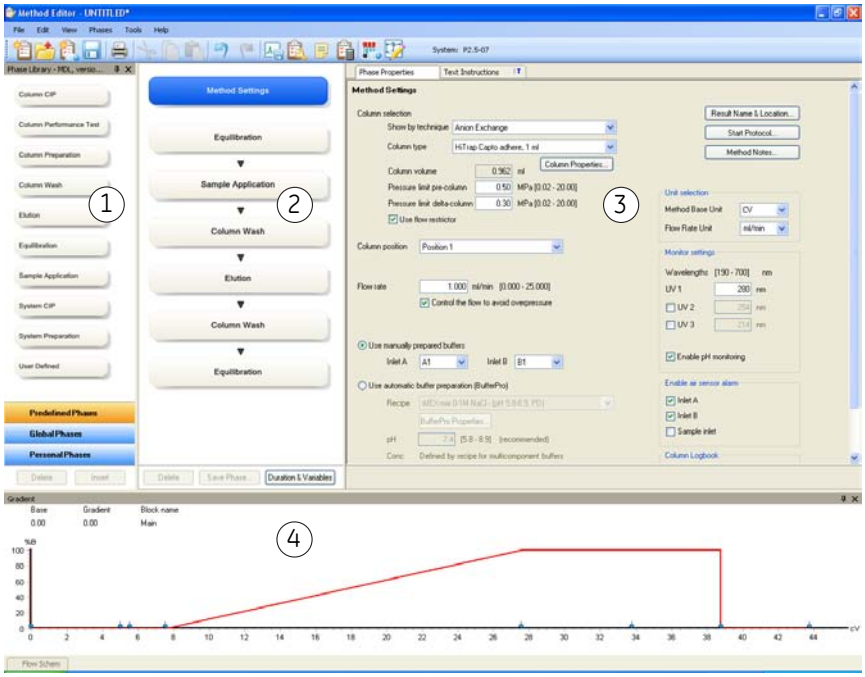
6.1.1 Create and edit methods

Introduction

Method creation in UNICORN 6 is performed in the ***Method Editor*** module. Predefined methods are available for a number of different chromatography techniques, for example affinity chromatography and gel filtration. There are also predefined methods for column preparation and system cleaning. A method is comprised of a number of phases. Each phase represents a major process step in the method, for example, equilibration or elution. If desired, a predefined method can be edited by adding, deleting and/or rearranging phases, and also by editing the settings of each phase included in the method.

Illustration of the Method Editor user interface

The illustration and table below shows the ***Method Editor*** module and its panes.



Part	Description
1	***Phase Library***: Displays all available phases.
2	***Method Outline***: Presents an overview of the phases included in the active method.
3	***Phase Properties***: Presents detailed settings for the selected phase.
4	***Gradient***: Illustrates the gradient used in the active method.

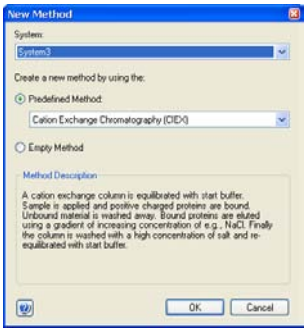
Create a method

Follow the instruction below to create and edit a chromatographic method based on a predefined method.

Step	Action
1	Open the ***Method Editor*** module and click the ***New Method*** icon.

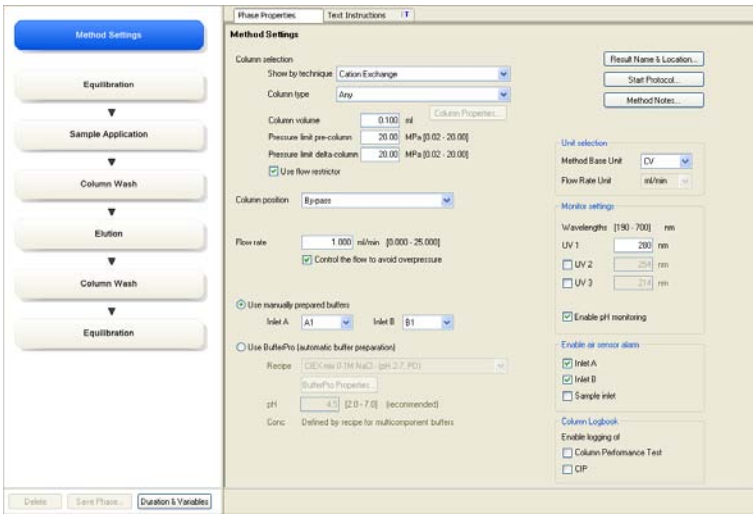
Result: The ***New Method*** dialog opens.

2	Select ***System*** and ***Predefined Method*** in the dialog. Click ***OK***.



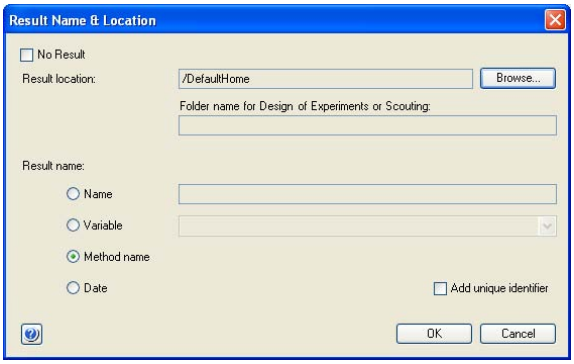
Result: The phases included in the chosen method show in the ***Method Outline*** pane, and the default settings for each of the phases show in the ***Phase Properties*** pane.

Step	Action
3	In the ***Phase Properties*** pane of the ***Method Settings*** phase, edit general settings like ***Column type*** and ***Method Base Unit***. UNICORN automatically calculates correct settings for volume, flow rate, and pressure limits based on the selected column type. The illustration below shows the ***Method Outline*** pane and the ***Phase Properties*** pane of the ***Method Settings*** phase.



Step	Action
4	In the ***Phase Properties*** pane of the ***Method Settings*** phase, click the ***Result Name & Location*** button to specify the name and location of the results from the method runs.

Result: The ***Result Name & Location*** dialog opens.

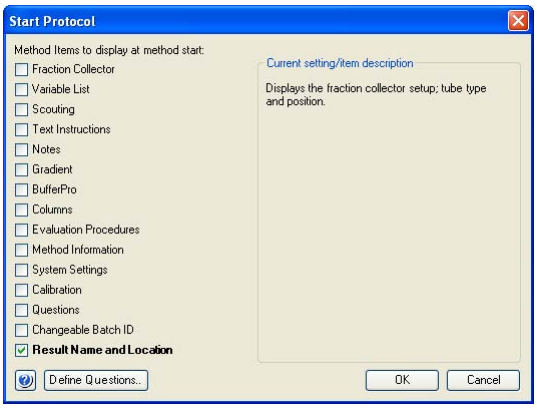


In the ***Result Name & Location*** dialog:

- Set ***Result location*** by clicking the ***Browse*** button and select a folder in which to save the results. By default, the results will be saved in your home folder.
- Select ***Result name***. With the default selection ***Method name*** the results will be named with the method name together with a serial number.
- Click ***OK*** to confirm and close the dialog.

Step	Action
5	In the ***Phase Properties*** pane of the ***Method Settings*** phase, click the ***Start Protocol*** button if you wish to include a start protocol.

Result: The ***Start Protocol*** dialog opens.

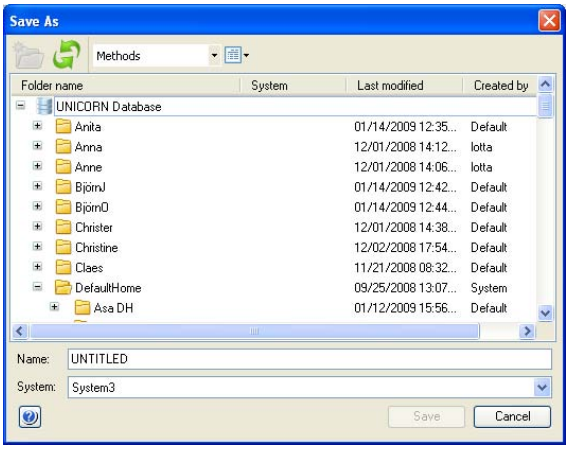
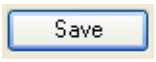


In the ***Start Protocol*** dialog:

- Select items to display at method start. ***Result Name and Location*** is selected by default.
- Click ***OK*** to confirm and close the dialog.

6	Select the next phase in the ***Method Outline*** pane by clicking it, and choose appropriate parameter values in the ***Phase Properties*** pane.
7	Repeat step 6 until all phases are edited.

TIP: If the instrument will not be used for a couple of days or longer, add one or several ***System CIP*** phases to the end of the method as described below.

Step	Action
8	Click the ***Save the method*** icon.  *Result:* The ***Save As*** dialog opens. 
9	In the ***Save As*** dialog: • Select a target folder to enable the ***Save*** button. • Type a ***Name*** for the method. • Select a ***System*** from the list. • Click the ***Save*** button.  *Result:* The created method is saved in the selected folder.

Add phases to a method

Follow the instruction below to add phases to a method.

Step	Action
1	Select the desired phase in the ***Phase Library*** pane.
2	Drag-and-drop the phase to requested position in the ***Method Outline*** pane. The phase can be placed at any postion below the ***Method Settings*** phase. *Result:* The phase is included in the method at the the requested position.

Delete phases from a method

Follow the instruction below to delete phases from a method.

Step	Action
1	Select the phase to delete in the ***Method Outline*** pane. ***Note:*** *The* ***Method Settings*** *phase cannot be deleted.*
2	Click the ***Delete*** button located below the ***Method Outline*** pane.  *Result:* The selected phase is excluded from the method.

Rearrange phases within a method

Follow the instruction below to rearrange phases within a method.

Step	Action
1	Select the phase to move in the ***Method Outline*** pane. ***Note:*** *The* ***Method Settings*** *phase cannot be moved.*
2	Drag-and-drop the phase to requested position in the ***Method Outline*** pane. *Result:* The selected phase is moved to the requested position.

Choose a maintenance method

A number of predefined methods for preparation and cleaning are available. Use these maintenance methods to prepare and clean the system and columns, and to fill the system and columns with storage solution. The table below gives suggestions for what methods and solutions to choose for the different purposes.

If you wish to...	Phase/Method	Solution
Prepare tubing and instrument modules before a run	System Preparation	suitable buffers
Prepare column before a run	Column Preparation	suitable buffers
Clean tubing and modules	System CIP	0.5-1 M NaOH
Clean column	Column CIP	0.5-1 M NaOH
Leave system in storage solution	System CIP	20% Ethanol
Leave column in storage solution	Column CIP	20% Ethanol

TIP: CIP (Cleaning-In-Place) and preparation of columns and system may be run either as separate predefined methods or as phases included in chromatographic methods.

TIP: The ***System CIP*** and ***System Preparation*** phases are designed to use one cleaning solution each. The ***System Preparation*** method includes two ***System Preparation*** phases and the ***System CIP*** method includes three ***System CIP*** phases. To use additional solutions sequentially, add phases to the method.

Note: *Usually 0.5-1 M NaOH is used for cleaning of the system and column. However, before choosing cleaning agent always consider the media and buffers used, and also the chemical resistance of the column.*

6.1.2 Predefined methods

The predefined methods that are available are described in the table below.

Method	Description
Affinity chromatography (AC)	After equilibration and sample application, the protein of interest is adsorbed to the column ligand. After a wash to remove unbound sample, elution is performed either by using a buffer containing a competitor to displace the protein of interest, or by changing the pH or ionic strength. Finally, the column is re-equilibrated with start buffer.
Anion Exchange Chromatography (AIEX)	After equilibration and sample application, negatively charged proteins are adsorbed to the column ligand. After a wash, to remove unbound sample, elution is performed using a gradient of increasing salt concentration (of e.g., NaCl). Finally, the column is washed and re-equilibrated with start buffer.
Cation Exchange Chromatography (CIEX)	After equilibration and sample application, positively charged proteins are adsorbed to the column ligand. After a wash, to remove unbound sample, elution is performed using a gradient of increasing salt concentration (of e.g., NaCl). Finally, the column is washed and re-equilibrated with start buffer.
Chromatofocusing (CF)	After equilibration and sample application, elution is performed using a pH gradient. The proteins separate and elute according to their isoelectric points. Finally, the column is re-equilibrated.
Column CIP	The column is filled with a cleaning solution. Select inlet positions. Enter the solution identity, volume, flow rate and incubation time. By adding steps, several cleaning solutions can be used. Suggestions for cleaning steps are available for a number of column types.
Column Performance Test	After equilibration of the column, sample is injected via a capillary loop and eluted isocratically. A non-adsorbing sample like acetone or salt should be used. After the run, calculate column performance in the Evaluation module. The efficiency of the column is determined in terms of height equivalent to a theoretical plate (HETP), and the peak asymmetry factor (A_s). The result is logged in the column logbook.
Column Preparation	The column is filled with buffer solution. Select inlet positions. Enter the solution identity, volume, flow rate and incubation time. By adding steps, several preparation solutions can be used.

Method	Description
Desalting (DS)	After equilibration and sample application, the proteins are eluted isocratically. This technique is commonly used for buffer exchange.
Gel filtration (GF)	After equilibration and sample application, proteins separate and elute according to their size (largest first).
Hydrophobic Interaction Chromatography (HIC)	After equilibration and sample application (use a buffer containing a high salt concentration, for example 2 M Ammonium Sulphate) hydrophobic proteins are adsorbed to the column ligand. After a wash to remove unbound sample, elution is performed using a gradient of decreasing salt concentration. Finally, the column is washed and re-equilibrated with start buffer.
Reversed Phase Chromatography (RPC)	After equilibration and sample application, hydrophobic proteins adsorb to the column ligand. After a wash to remove unbound sample, elution is performed by generating a gradient of a non-polar, organic solvent such as Acetonitrile. Finally, the column is washed and re-equilibrated.
System CIP	The system is filled with cleaning solution. Select for example inlets, outlets and column positions to be cleaned. Three ***System CIP*** phases are included in the method to facilitate the use of three different cleaning solution. Additional ***System CIP*** phases can be added from the ***Phase Library*** if desired.
System Preparation	The system is filled with preparation solution. Select for example inlets, outlets and column positions to be prepared. Two ***System Preparation*** phases are included in the method. Additional ***System Preparation*** phases can be added from the ***Phase Library*** if desired.

6.1.3 Predefined phases

The predefined phases that are available in the predefined methods and in the ***Phase Library*** are described in the table below.

Phase	Description
Method Settings	The first, and mandatory, phase in any method. Defines common parameters used in the subsequent phases.
Equilibration	Equilibrates the column before purification, or re-equilibrates the column after purification.
Sample Application	Applies sample to the column. Defines the sample application technique, the sample volume, and the handling of flowthrough.
Column Wash	Washes out unbound sample after sample application or removes strongly bound proteins after elution.
Elution	Elutes the sample from the column. Defines parameters for the elution and fractionation settings.
Column Preparation	Prepares the column before use by removing the storage solution and equilibrating the column. By adding steps, several preparation solutions can be used sequentially.
Column CIP	Cleans the column after purification runs by rinsing the column with a cleaning solution to remove unspecifically bound proteins. By adding steps, several cleaning solutions can be used sequentially.
System Preparation	Prepares the system before a run by removing storage solution and filling the system and inlets with buffer solution. One preparation solution is used per phase.
System CIP	Cleans the system after purification runs by rinsing the system with a cleaning solution. One cleaning solution is used per phase.
Column Performance Test	Tests the efficiency of a packed column in terms of height equivalent to a theoretical plate (HETP), and the peak asymmetry factor (A_s).

6.2 Print a method

Introduction

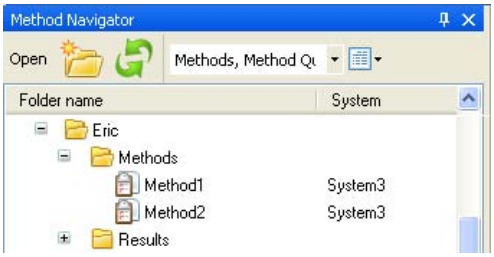
This section describes how to print the text instructions and variables of a method. UNICORN uses the printers and printer settings that are installed on your computer.

How to print a method

Follow the instruction below to print a method.

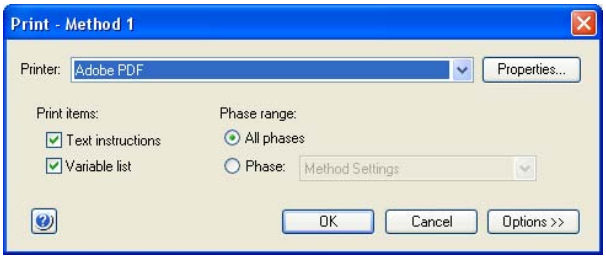
Step	Action
1	Open the ***Method Editor*** module and click the ***Open Method Navigator*** icon in the toolbar.

Result: The ***Method Navigator*** pane opens.



2	Select the method to print and click the ***Open a Method*** icon in the navigator toolbar.

Result: The selected method opens.

Step	Action
3	Click the ***Print*** icon.  *Result:* The ***Print*** dialog opens. 
4	In the ***Print*** dialog: • select ***Printer*** • click ***OK*** *Result*: The method is printed.

7 Prepare the system for a run

Introduction

Follow this chapter to perform the necessary preparations of the system before starting a run.

WARNING

Always use appropriate personal protective equipment during operation and maintenance of ÄKTAavant system.

Contents

This chapter contains the following sections:

7.1 Before you prepare the system

Introduction

It is important to prepare the system in accordance with the settings in the method to be run. Before preparing the system, check the settings in the ***Method Editor*** and make sure that all accessories to be used are available.

Checklist

Make sure to prepare the system in accordance with the settings in the method to be run. Remember to check:

- which valve ports to use for inlets and outlets
- which cassettes with corresponding deep well plates and/or tubes to use in the Fraction collector, if applicable
- which column type to use
- which column position to use
- which sample application technique to use
- that the pH electrode is connected, if applicable
- if it is a reversed phase chromatography (RPC) run

WARNING

RPC runs with 100% acetonitrile. Always replace the green PEEK tubing between the used system pump and the pressure monitor with orange PEEK tubing, i.d. 0.5 mm, before running RPC with 100% acetonitrile. Set the System pressure alarm to 10 MPa.

WARNING

Fraction collector. Do *not* fractionate flammable liquids. When running RPC methods, or other procedures using solvent based buffers, collect fractions through the outlet valve.

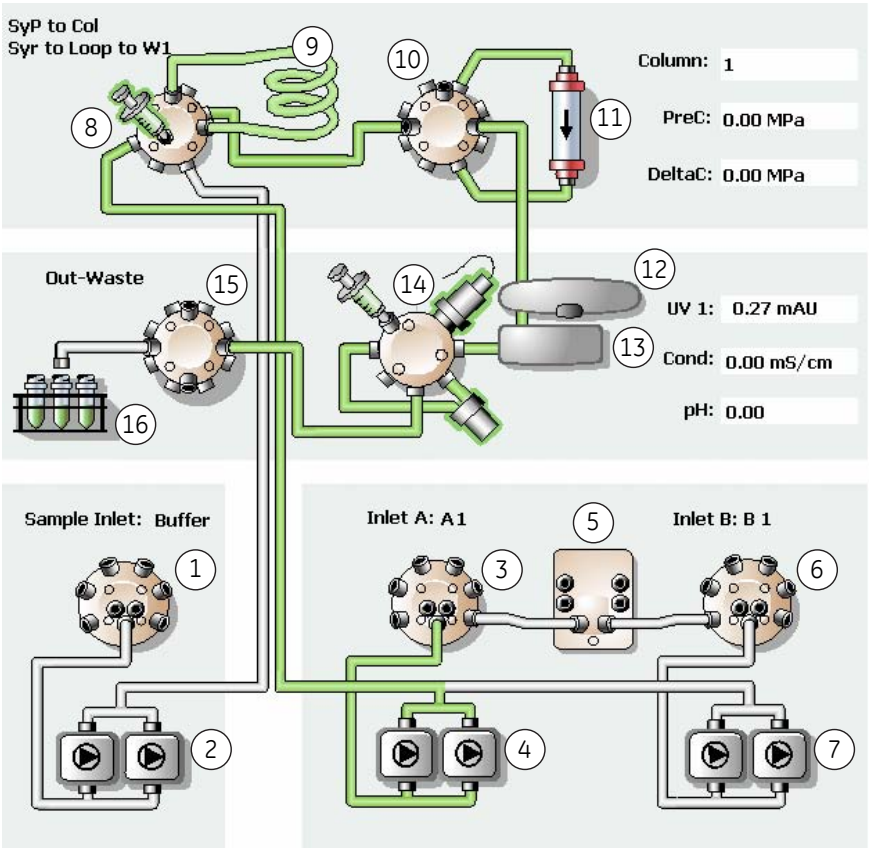
7.2 Prepare the flow path

Introduction

The flow path contains tubing, valves, pumps and monitors. This section gives an overview of the flow path and describes how to prepare the flow path before a run.

Illustration of the flow path

The illustration below shows the flow scheme as illustrated in the ***System Control*** module. The individual instrument modules are presented in the table below.



Part	Description
1	Sample inlet valve
2	Sample pump
3	Inlet valve A
4	System pump A
5	Quaternary valve
6	Inlet valve B
7	System pump B
8	Injection valve
9	Capillary loop or Superloop
10	Column valve
11	Column
12	UV monitor
13	Conductivity monitor
14	pH valve with pH monitor and flow restrictor
15	Outlet valve
16	Fraction collector

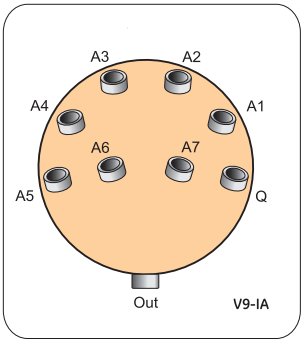
Tubing and connectors

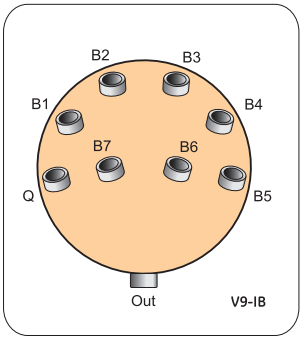
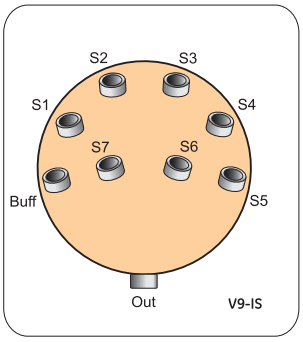
The table below shows what types of tubing and connectors to use for different purposes.

Scope of use	Tubing	Connector	Photo
Inlet tubing	Teflon™, o.d. 1/8", i.d. 1.6 mm	Tubing connector, 1/8" + Ferrule (yellow), 1/8"	
Tubing to connect columns	PEEK, o.d. 1/16", i.d. 0.50 mm	Fingertight connector, 1/16"	
Waste tubing	Tefzel™, o.d. 1/8", i.d. 1.0 mm	Fingertight connector, 1/16"	

Inlet ports

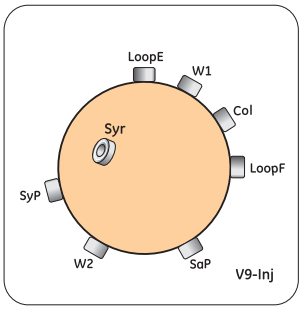
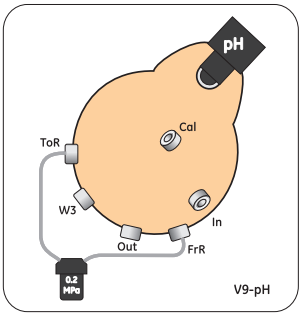
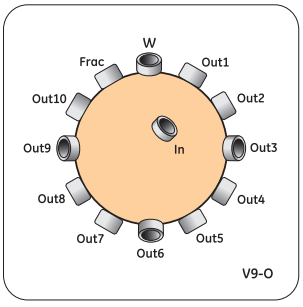
The table below shows the inlet ports of Inlet valve A, Inlet valve B and Sample inlet valve.

Valves and ports	Illustrations
Inlet valve A Inlet ports: • **A1-A7**	

Valves and ports	Illustrations
Inlet valve B Inlet ports: • **B1-B7**	
Sample inlet valve Inlet ports: • **S1-S7, Buff**	

Waste ports

The table below shows the waste ports of Injection valve, pH valve, and Outlet valve.

Valves and ports	Illustrations
Injection valve Waste ports: • **W1, W2**	
pH valve Waste port: • **W3**	
Outlet valve Waste port: • **W**	

Prepare inlets and outlets

Follow the instruction below to prepare the inlets and outlets that are to be used in the method run.

Step	Action
1	Connect inlet and outlet tubing to the ports that are to be used.
2	Immerse all inlet tubing that is to be used during the method run in the correct buffers.
3	Immerse the waste tubing from the Injection valve, the pH valve, the Outlet valve, and the Fraction collector into a sufficiently large waste vessel (typically 2-10 liters).

7.3 Prime buffer inlets and purge System pumps

Introduction

Before usage of the System pumps, it is important to:

1 Prime the inlets (fill the buffer inlets with liquid).

2 Purge the System pumps (remove air from the pumps).

Prime buffer inlets

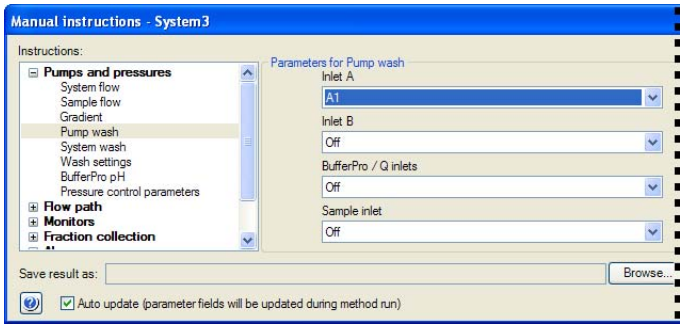
Follow the instruction below to prime the buffer inlets. Each buffer inlet that is to be used during the method run has to be primed.

Step	Action
1	Make sure that all buffer inlet tubing that is to be used during the method run is immersed in the correct buffers.
2	Make sure that the waste tube connected to Injection valve port **W1** is immersed in a waste vessel.
3	Open the ***System Control*** module in UNICORN, and select ***Manual:Execute Manual Instructions***. *Result:* The ***Manual instructions*** dialog opens.

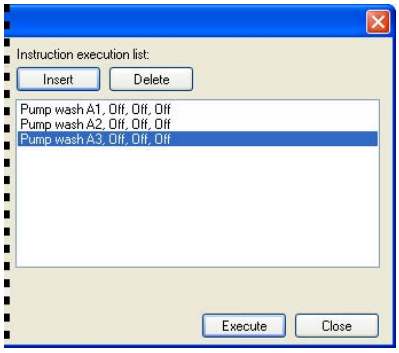
Step	Action
4	**TIP:** If desired, the flow rate and volume of the pump wash can be edited before the pump wash is started. In the ***Manual instructions*** dialog, select ***Pumps and pressures:Wash settings*** and edit the settings for ***System flow rate*** and ***System pump wash volume***. Click ***Execute***.

In the ***Manual instructions*** dialog:

- Select ***Pumps and pressures:Pump wash***, and select the inlet to be primed from the ***Inlet A*** or ***Inlet B*** drop-down list.



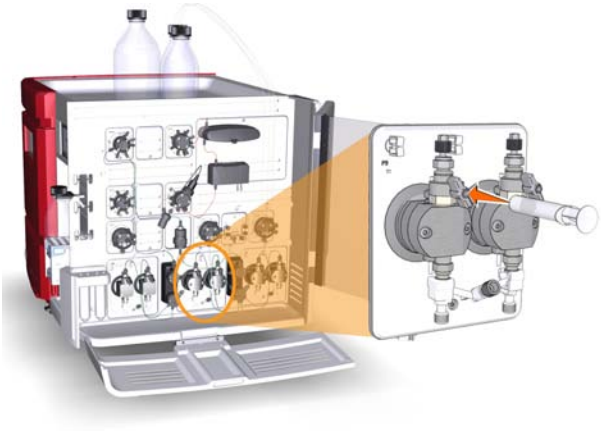
- If several inlets are to be used, click ***Insert*** to insert a selected inlet in the ***Instruction execution list***. Insert all inlets to be used in the ***Instruction execution list***.



- Click Execute.

Result: A pump wash of the first inlet in the ***Instruction execution list*** starts.

Step	Action
5	Connect a 25-30 ml syringe to one of the purge valves of the selected system pump. Make sure that the syringe fits tightly into the purge connector.



6	Open the purge valve by turning it counterclockwise about 3/4 of a turn. Draw 5-10 ml of liquid slowly into the syringe.
7	Close the purge valve by turning it clockwise. Disconnect the syringe and discard its contents.
8	Click the **Continue** button on the Instrument display to start a pump wash of the next inlet in the ***Instruction execution list***.
9	Repeat steps 4-7 until all inlets in the ***Instruction execution list*** have been primed (filled with liquid).

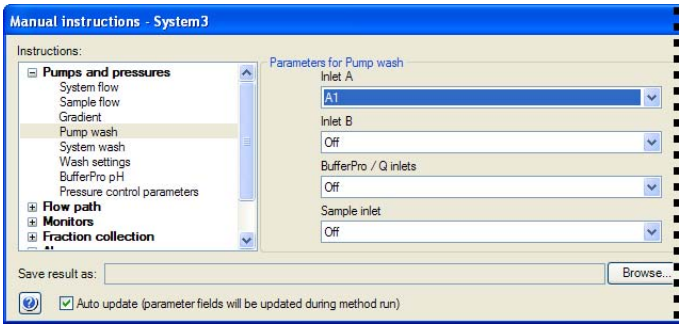
Purge the system pumps

Follow the instruction below to purge System pump A and/or System pump B. Note that both pump heads of each system pump have to be purged. Also, note that both system pumps have to be purged after priming of buffer inlets.

TIP: Prime all buffer inlets and purge System pump B before finally purging System pump A with start buffer. The instrument is then ready for start of a method run.

Step	Action
1	In the ***Manual instructions*** dialog:

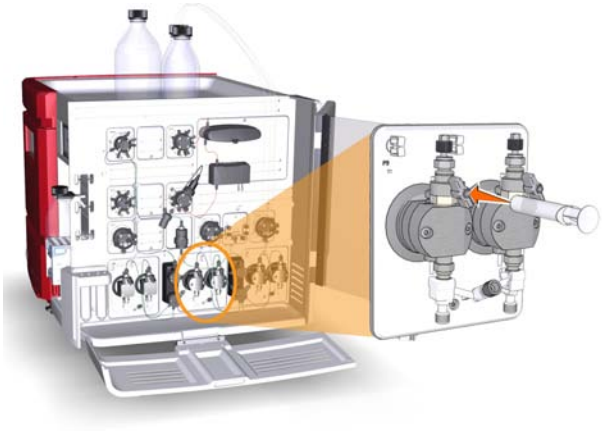
- Select ***Pumps and pressures:Pump wash***, and select one inlet from the ***Inlet A*** or ***Inlet B*** drop-down list. Preferably, choose the buffer inlet to be used in the beginning of the method run.



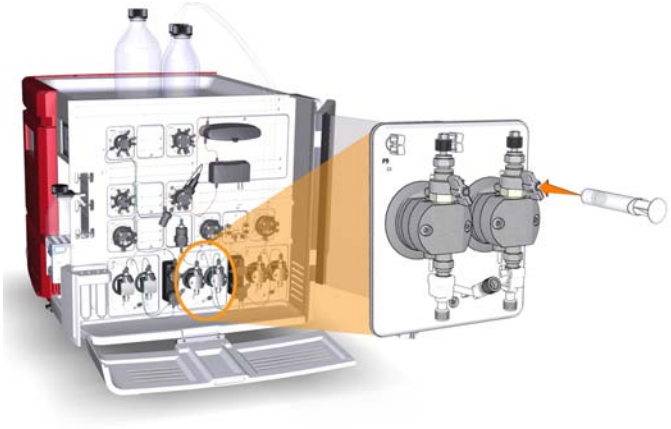
- Click Execute.

Result: A pump wash of the selected inlet starts.

Step	Action
2	Connect a 25-30 ml syringe to the left purge valve of the selected system pump. Make sure that the syringe fits tightly into the purge connector.



3	Open the purge valve by turning it counterclockwise about 3/4 of a turn. Draw 5-10 ml of liquid slowly into the syringe with a rate of about 1 ml/s.
4	Close the purge valve by turning it clockwise. Disconnect the syringe and discard its contents.
5	Connect the syringe to the purge valve on the right pump head of the selected system pump, and repeat steps 3-4.



7.4 Connect a column

Introduction

This section describes how to connect a column to the instrument, using a column holder and without introducing air into the flow path. Several types of column holders are available for ÄKTAavant.

WARNING

Before connecting a column to the ÄKTAavant instrument, read the instructions for use of the column. To avoid exposing the column to excessive pressure, make sure that the pressure limit is set to the specified maximum pressure of the column.

Methods automatically include a pressure alarm based on the tolerance of the chosen column type. However, when running manual runs you have to set the pressure limits yourself.

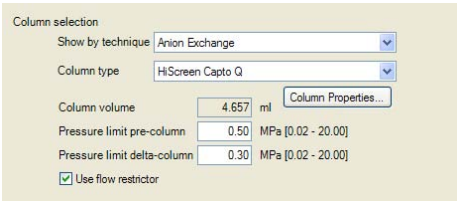
CAUTION

Do not overtighten when connecting columns. Overtightening might rupture the connectors or squeeze the tubing and thereby result in high backpressure.

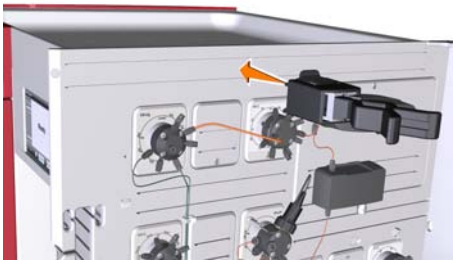
Attach a column holder and connect a column to the instrument

Follow the instruction below to connect a column to the instrument. Always use a column holder. The column is connected to two opposite parts of the Column valve, using appropriate tubing and connectors.

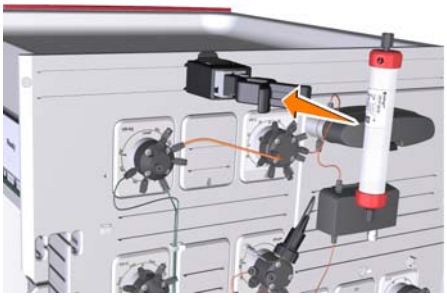
Step	Action
1	Choose a column according to the column selections made in the method to be run.

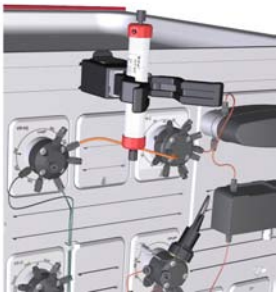


2 Attach an appropriate column holder to the rail on the instrument.



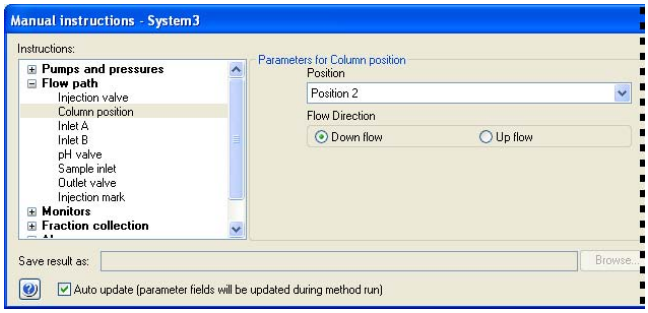
3 Attach the column to the column holder.





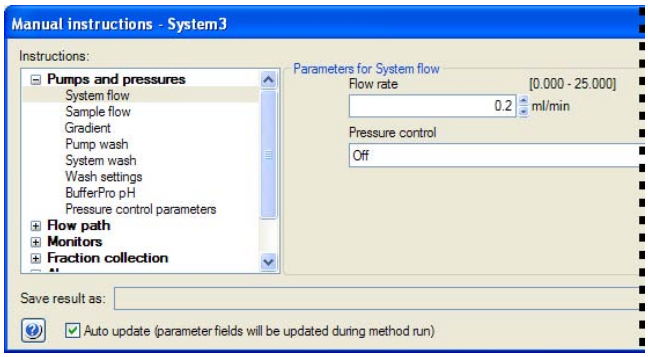
Step	Action
4	Connect a suitable tubing to a Column valve port, for example port **2A** if column position 2 was chosen in the method to be run.

5	Open the ***System Control*** module and select ***Manual:Execute Manual Instructions***. *Result*: The ***Manual instructions*** dialog opens.
6	In the ***Manual instructions*** dialog: • Select ***Flow path:Column position***. • Choose the ***Position*** used for connection of the column, in this example ***Position 2***. • Choose the ***Flow Direction*** Down flow.



• Click Execute.

Result: The Column valve switches to position 2.

Step	Action
7	In the ***Manual instructions*** dialog: • Select ***Pumps and pressure:System flow***. • Enter a low ***Flow rate*** (e.g., 0.2 ml/min).  • Click Execute. *Result:* A system flow of 0.2 ml/min starts.
8	When buffer leaves the tube on port **2A** in a continuous mode and the top part of the column is filled with buffer, connect the tube to the top of the column. 

Step	Action
9	Connect a tube to the bottom of the column.

10	When buffer leaves the tube at the bottom of the column in a continuous mode, connect this tube to the Column valve. Use the port opposite to the one already connected to the column, in this example port **2B**.

11	Click the ***End*** icon in the ***System Control*** toolbar to end the run.

7.5 Calibrate the pH monitor

Introduction

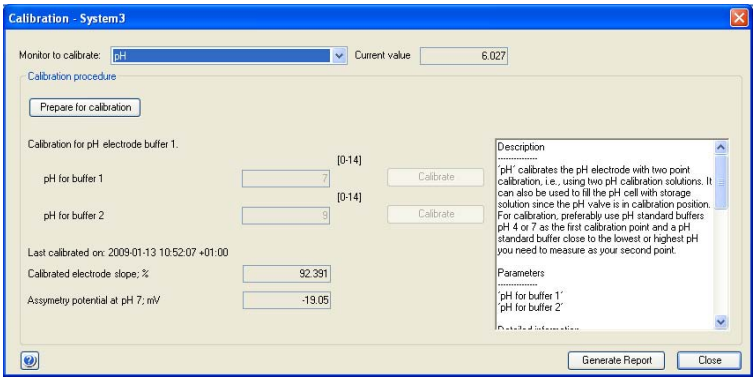
If pH will be measured during the chromatographic run, the pH monitor should be calibrated before the run is started. Use two pH calibration buffers with a difference of at least one pH unit. Preferably use a pH standard buffer pH 4 or 7 as the first calibration point, and a pH standard buffer close to the lowest or highest pH you need to measure as your second point. Allow the buffers to reach the operating temperature before use.

Note: *Do not run a system flow during pH calibration.*

Calibrate the pH monitor

Follow the instruction below to perform the calibration.

Step	Action
1	Open the ***System Control*** module and select ***System:Calibrate***. *Result*: The ***Calibration*** dialog opens.
2	Set the pH monitor as the ***Monitor to calibrate*** by selecting ***pH*** from the list.
3	Click Prepare for calibration. *Result*: The pH valve switches to the calibration position.
4	Enter the pH of the first pH standard buffer in the ***pH for buffer 1*** field.



Step	Action
5	Fill a syringe with approximately 10 ml of the first pH standard buffer. Connect the syringe to the Luer connector in pH valve port **Cal**, and inject the buffer.
6	When the ***Current value*** is stable, click Calibrate.
7	Wash the pH flow cell by injecting water into pH valve port **Cal** using a new syringe.
8	Enter the pH of the second pH standard buffer in the ***pH for buffer 2*** field.
9	Repeat steps 5-6 using the second pH standard buffer. *Result:* The calibration date and time are displayed in the dialog, and also values for ***Calibrated electrode slope*** and ***Asymmetry potential at pH 7***.
10	Is the ***Calibrated electrode slope*** ≥ 80% and the ***Asymmetry potential at pH 7*** inside the interval ±60 mV? • If Yes: Click Close to switch the pH valve back to the default position, and to close the ***Calibration*** dialog. • If No: Clean the pH electrode, and repeat the calibration procedure. If this does not help, replace the electrode. For information about cleaning and replacing the pH electrode, see User Manual *Chapter Maintenance*.

CAUTION

pH-electrode. Handle the pH-electrode with care. The glass tip may break and cause injury.

7.6 Prepare the Fraction collector

Introduction

This section describes how to prepare the Fraction collector. For information regarding the types of deep well plates, tubes and cassettes, see *ÄKTAavant and UNICORN 6 User Manual*.

WARNING

Fraction collector. Do *not* fractionate flammable liquids. When running RPC methods, or other procedures using solvent based buffers, collect fractions through the outlet valve.

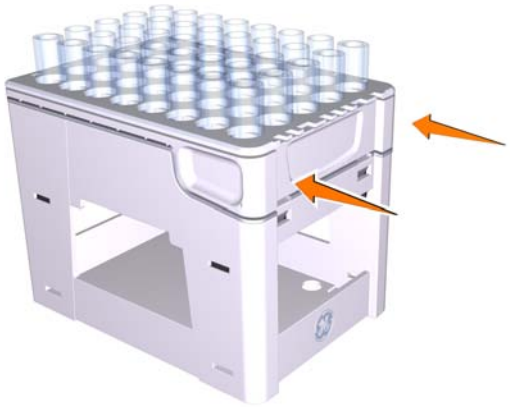
Prepare the Fraction collector

Follow the instruction below to prepare the Fraction collector before a run. Choose deep well plates and/or tubes according to the fractionation settings in the method to be run, and choose appropriate cassettes.

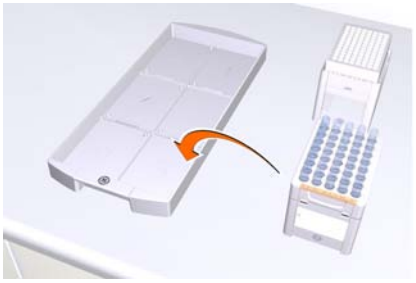
Step	Action
1	If you are to use cassettes with the QuickRelease function, open the cassettes.

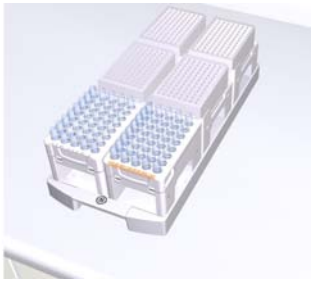
Step	Action
2	Place the tubes and deep well plates in the cassettes. Make sure that the deep well plates are rotated so that the well marked A1 is positioned above the A1 marking on the cassette.

3	Close the cassettes that have the QuickRelease function.

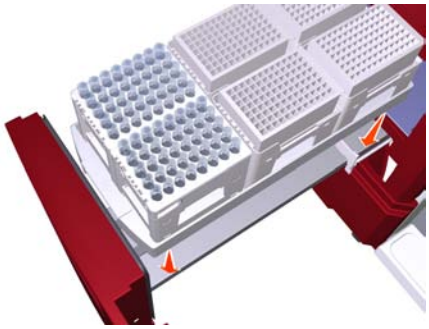


Step	Action
4	Place the cassettes on the Cassette tray. Make sure that the Cassette type code (see illustration below) faces the front of the tray marked with the GE logo.





5	Open the Frac drawer by pressing the handle upwards, and pulling out the drawer.

Step	Action
6	Place the Cassette tray on the Tray support of the Frac drawer. Make sure that the front of the tray (marked with the GE logo) faces the front of the drawer and is hooked onto the two pins.
	
7	Close the Frac drawer. Make sure that it snaps into closed position.

Cassette identification

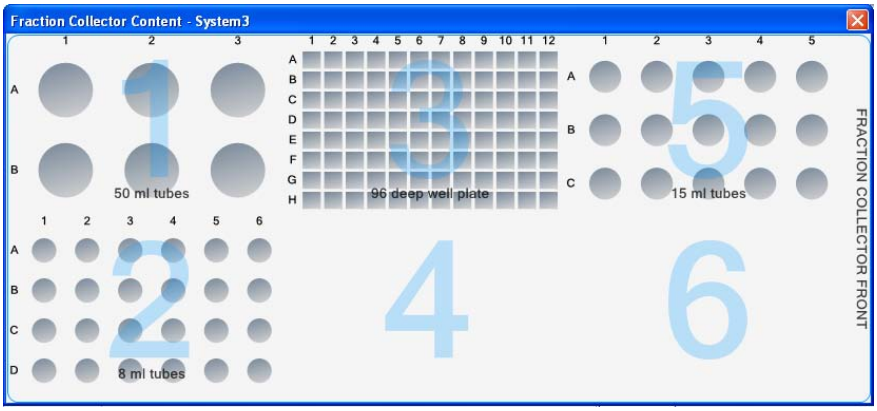
After the Frac drawer has been closed, the Frac arm scans the Cassette type code of each cassette to identify the cassette types. If deep well plates are used, the instrument also identifies the types of deep well plates.

WARNING

Moving parts in Fraction collector. Do not open the Fraction collector drawer when the Fraction collector is active. If you need to access the Fraction collector, press ***Pause***, and make sure that the movement has stopped before opening the drawer.

View Fraction collector content

To view the content of the Fraction collector, open the ***System control*** module and select ***View:Fraction Collector Content***.



8 Run a method

Introduction

This chapter describes how to start up and run a method, and also how to handle the system after the run.

Contents

This chapter contains the following sections:

8.1 Before you start

Introduction

Before starting a run, it is necessary to read and understand the information in this section and to perform the checks listed below.

Checklist

Make sure that the system is correctly prepared. Check that:

- the system is prepared according to chapter 6.
- a suitable column has been selected for the application (consider target protein and pressure range).
- the buffer inlet tubing is immersed in correct buffer vessels (consider solution identity and volume).
- the waste tubing is immersed into appropriate waste vessels (consider vessel size and vessel material).
- no tubing is twisted and the flow path is free from leakage.

Hold, pause or stop the run

At the end of a method the run stops automatically. All pumps stop and the system makes an acoustic end signal and displays ***End*** in the ***Run Log***.

To interrupt a method during a run you may use the ***Hold***, ***Pause*** or ***End*** icons in ***System Control***. A held or paused method run can be resumed by using the ***Continue*** icon. See the instructions in the table below.

If you want to...	then...
temporarily hold the method, with current flow rate and valve positions sustained	click the ***Hold*** icon. 
temporarily pause the method, and stop all pumps	click the ***Pause*** icon. 

If you want to...	then...
resume, for example, a held or paused method run.	click the ***Continue*** icon.  ***Note:*** *An ended method cannot be continued.*
permanently end the run	click the ***End*** icon. 

Note: *When ending a method run in advance, it is possible to save the partial result.*

Warnings concerning use of hazardous substances

WARNING

Hazardous chemicals during run. When using hazardous chemicals, run ***System CIP*** and ***Column CIP*** to flush the entire system tubing with distilled water, before service and maintenance.

WARNING

Hazardous biological agents during run. When using hazardous biological agents, run ***System CIP*** and ***Column CIP*** to flush the entire system tubing with bacteriostatic solution (e.g., NaOH) followed by a neutral buffer and finally distilled water, before service and maintenance.

8.2 Applying the sample

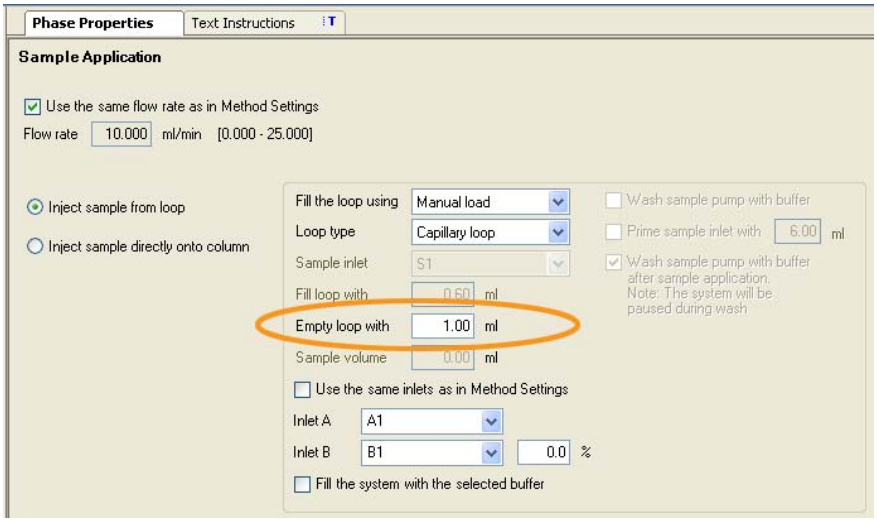
Introduction

A number of different sample application techniques are available. The sample can be applied either directly onto the column using the Sample pump, or via a loop. A loop can be filled either manually or by use of the Sample pump. This section describes sample application using a syringe to manually fill a capillary loop. The two stages of the sample application are described in the table below. For detailed instructions and information regarding the different sample applications techniques, see *User Manual*.

Stage	Description
Load	The capillary loop is filled with sample.
Inject	The sample is injected onto the column.

Sample application through a capillary loop

A capillary loop is manually filled with sample using a syringe connected to the Injection valve port **Syr**. During the method run, the sample is automatically injected onto the column. The loop is emptied and washed out using buffer from the system pumps. The total buffer volume to be used for emptying and washing the capillary loop is set in the ***Phase Properties*** tab of the ***Sample Application*** phase at ***Empty loop with***.



TIP: Empty the capillary loop with a buffer volume that exceeds the volume of the loop. This will ensure that the loop is completely emptied.

How to fill a capillary loop

Follow the instruction below to fill the capillary loop with sample.

Step	Action
1	Connect a suitable capillary loop to Injection valve ports **LoopF** (fill) and **LoopE** (empty).

2	Fill a syringe with sample.
3	Connect the syringe to the Injection valve port **Syr**.

Step	Action
4	Load sample into the capillary loop. To avoid sample loss due to siphoning, leave the syringe in the port until the sample has been injected onto the column during the run. **TIP:** It is recommended to overload the loop to make sure that the loop is completely filled. Excess of sample will leave the valve through port **W1**.

8.3 Start a method run

Introduction

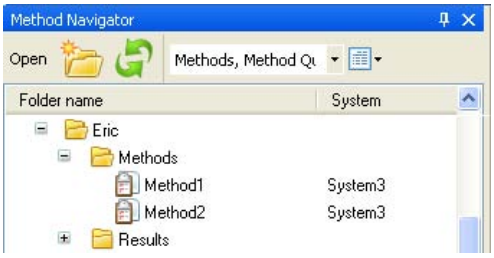
This section describes how to start a run using a previously created method. If ***Column Logbook*** was enabled during installation of the software, registration and selection of individual columns is possible at method start. The ***Column Logbook*** function includes, for example, logging of column history. For further information on column handling, please refer to *UNICORN 6 Method Manual*.

Choose and start a method

The instruction below describes how to open a method and start a run.

Step	Action
1	Open the ***System Control*** module and click the icon ***Open Method Navigator***.

Result: The ***Method Navigator*** pane opens.

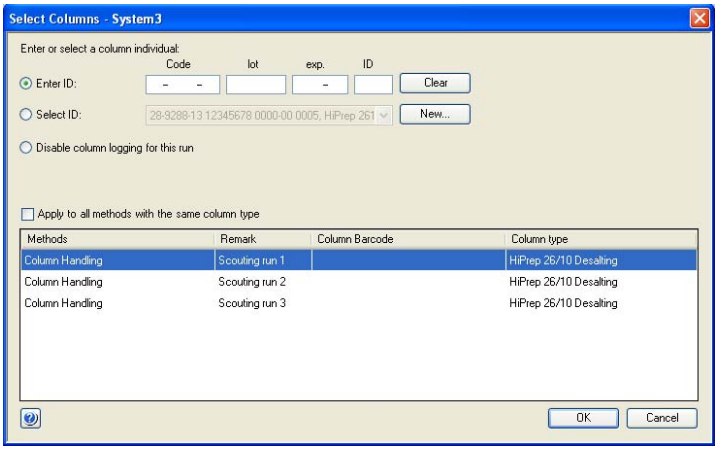


2	Select the method to run, and click the ***Run*** icon.

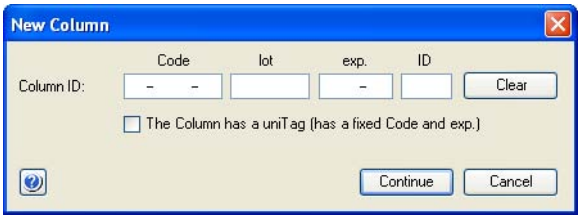
Result: The ***Start Protocol*** dialog opens.

3	Step through the displayed pages in the ***Start Protocol***, add requested input and make appropriate changes if necessary. Click ***Next***.

Step	Action
4	Click ***Finish*** on the last page of the ***Start Protocol***. *Result*: • If column logging was chosen at installation of UNICORN and a column type was selected at method creation, the ***Select Columns*** dialog opens. Continue with steps 5-9.



	• If column logging was *not* chosen at installation of UNICORN and/or *no* column type was selected at method creation, the run starts directly.
5	Is the column to be used already registered? • If No, continue to step 6. • If Yes, continue to step 9.
6	In the ***Select Columns*** dialog, click ***New***. *Result:* The first ***New Column*** dialog opens.

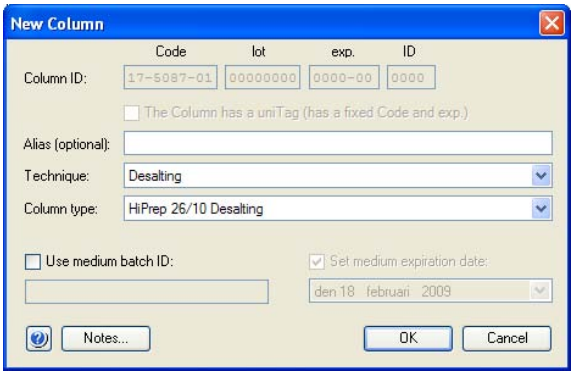


Step	Action
7	Register the column using the 2D Barcode scanner as follows:

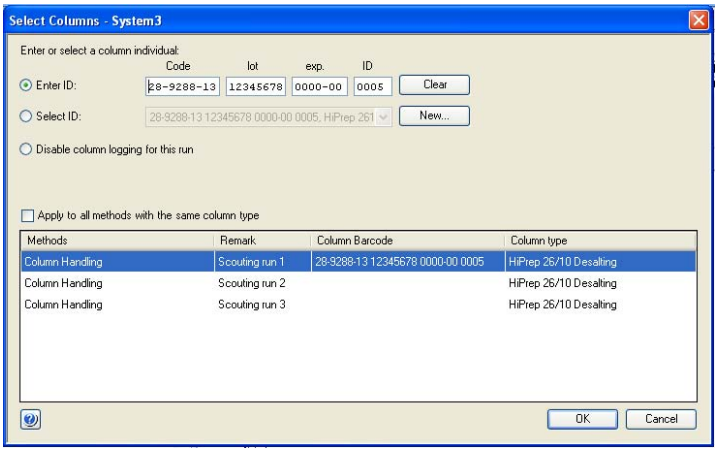
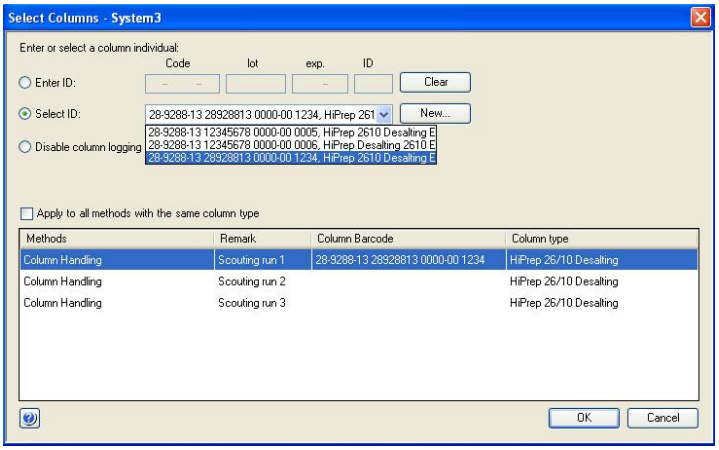
- Make sure that the pointer is placed in the first position of the ***Code*** field.
- Point the 2D Barcode scanner towards the data matrix tag on the column.
- Press and hold the trigger to create a beam.
- When the 2D Barcode scanner beeps, the column ID is registered and displayed in the dialog.

- Alternatively, manually enter the column ID, that you find on the column label, in the dialog using your keyboard.
- Click ***Continue***.

Result: The expanded ***New Column*** dialog opens.



Step	Action
8	In the expanded ***New Column*** dialog: • enter ***Alias*** (optional) • select ***Technique*** and ***Column type*** from the drop-down lists. • click ***OK***. **TIP:** Alias can be used for easy identification of a column. *Result:* The entered information is saved and the dialog closes.

Step	Action
9	In the ***Select Columns*** dialog: • Select ***Enter ID***. • Use the 2D Barcode scanner (see step 7) to enter the column ID.  • Alternatively, choose ***Select ID*** and select the column individual to be used in the run from the drop-down list.  • Click ***OK***. *Result:* The run starts. All necessary actions occur automatically according to the method, including ending of the run.

8.4 Monitor the run

Introduction

You may follow the on-going method run in the ***System Control*** module. The current system status is shown in the ***System state*** panel in the ***Run Data*** pane. For example, it may state ***Run***, ***Wash*** or ***Hold***. The same information is also shown on the Instrument display.

This section describes the data shown in ***System Control*** during a run, the layout of the module and the procedure to customize the view of the different panes.

Illustration of the System Control user interface

In the ***System Control*** module, four panes show by default (see illustration and table below).

Part	Description
1	***Run Data***: Presents current run data values.
2	***Chromatogram***: Illustrates data as curves.
3	***Run Log***: Presents all registered actions.
4	***Flow Scheme***: Illustrates the current flow path.

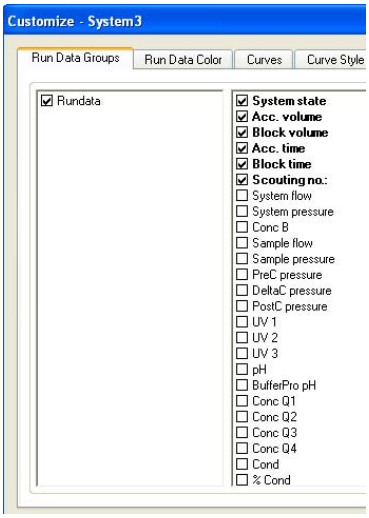
Customize Run Data

The ***Run Data*** pane shows real-time data from available monitors during the run.

To change what parameters to display in the pane:

- Click the ***Customize*** icon to open the ***Customize*** dialog.

- Under the ***Run Data Groups*** tab, select the parameters to display.
- Click ***OK*** to close the dialog.



Customize Chromatogram

The ***Chromatogram*** pane displays a graphical presentation of registered data from available monitors during the run.

To change the chromatogram properties:

- Click the ***Customize*** icon to open the ***Customize*** dialog.

- Make appropriate selections under the tabs described in the table below.

Tab	Customization
Curves	Select curves to be displayed.
Curve style and color	Customize the appearance of the displayed curves.
X-axis	Select base unit (time or volume) and set X-axis scale.
Y-axis	Select which Y-axes to display and set Y-axis scale for the different curves.

- Click ***OK*** to close the dialog.

Note: *These operations determine which curves are displayed and their appearance in the* ***Chromatogram*** *pane and on printouts. The original raw data curves can never be modified or removed from the result.*

View details in Chromatogram

Follow the instructions below to view selected parts of the chromatogram.

If you want to...	then...
identify a curve	position the mouse pointer over the curve of interest. *Result*: The curve name is displayed.
select which curve the Y-axis scale refers to	click the curve of interest. *Result*: The Y-axis scale changes to the unit applicable for the curve.
zoom in the curves	select the area of interest by moving the pointer with the left mouse button held down. *Result*: The selected area is enlarged.
zoom out again	click the right mouse button and select ***Reset Zoom***. *Result*: The original area is restored in one step.

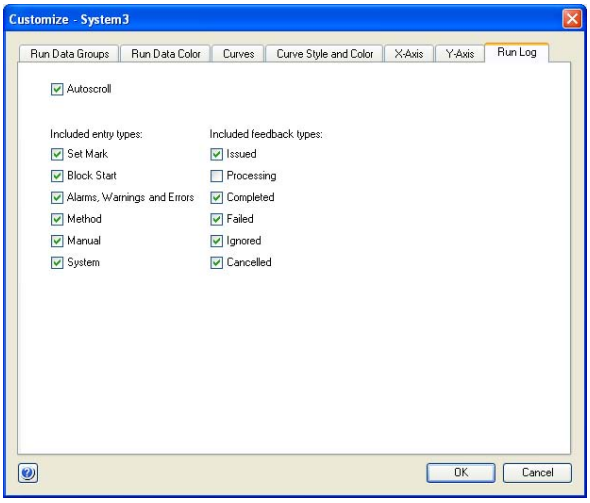
Customize Run Log

The ***Run Log*** pane displays all registered actions during the run. Scroll up to see the entire log.

To change what items to display in the pane:

- Click the ***Customize*** icon to open the ***Customize*** dialog.

- Under the ***Run Log*** tab, select the items to display.



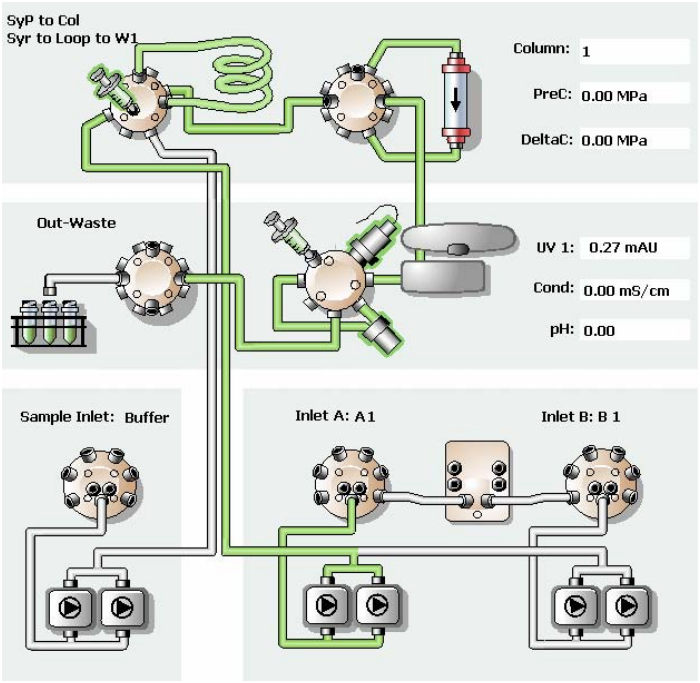
- Click ***OK*** to close the dialog.

Note: *These operations determine which data are shown in the **Run Log** pane during a run. All original raw data are saved and can never be modified or removed from the result.*

Flow scheme

The ***Flow Scheme*** pane displays the current flow path during the run. Color indication is applied, as shown in the table below. Real-time data from monitors are also displayed in the flow scheme. See illustration below.

Color	Indication
Green	Open flow path
Grey	Closed flow path
Red (not shown)	Alarm



8.5 After run procedures

Introduction

This section describes how to clean the instrument and columns after a chromatographic run, and how to prepare the system for storage.

The instrument and the columns should be cleaned between the runs. This will prevent, for example, sample contamination, protein precipitation and column clogging. If the instrument is not going to be used for a couple of days or longer, the instrument, columns and the pH flow cell should be filled with storage solution. For further information about cleaning and maintenance procedures, see *User Manual*.

TIP: To clean and fill the instrument and columns with storage solution, use ***System CIP*** and ***Column CIP*** either as separate, predefined methods or as phases included in a chromatographic method.

WARNING

Hazardous chemicals during maintenance. When using hazardous chemicals for system or column cleaning, wash the system or columns with a neutral solution in the last phase or step.

System cleaning

After a method run is completed, perform the following:

- Rinse the instrument with one or several cleaning solution(s) (e.g., NaOH, buffer solution or distilled water) using ***System CIP***.
- Empty the Fraction collector.
- Clean all spills on the instrument and on the bench using a moist tissue.
- Empty the waste vessel.
- Check that the pH electrode is in appropriate buffer.

System storage

If the instrument is not going to be used for a couple of days or longer, also perform the following:

- Fill the system and inlets with storage solution (e.g., 20% ethanol) using ***System CIP***.

Column cleaning

After a method run is completed, perform the following:

- Clean the column with one or several cleaning solution(s) using ***Column CIP***.

Column storage

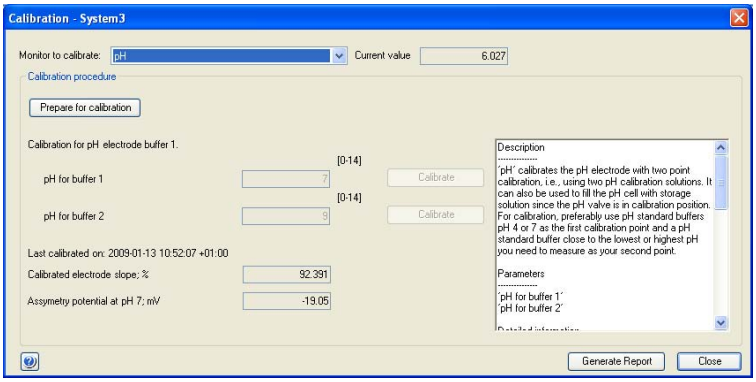
If the column is not going to be used for a couple of days or longer, also perform the following:

- Fill the column with storage solution (e.g., 20% ethanol) using ***Column CIP***.

pH electrode storage

Follow the instruction below to fill the pH flow cell with storage solution. The calibration function is used to switch the pH valve. However, no calibration is performed.

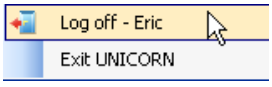
Step	Action
1	Open the ***System Control*** module and select ***System:Calibrate***. Result: The ***Calibration*** dialog opens.
2	In the ***Calibration*** dialog, select ***pH*** from the ***Monitor to calibrate*** drop-down list.
3	Press Prepare for calibration. *Result*: The pH valve switches to the calibration position.

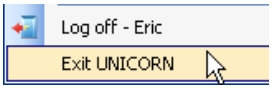


Step	Action
4	Prepare at least 10 ml storage solution by mixing equal volumes of a standard buffer pH 4 and a 1 M Potassium Nitrate (KNO_3) solution.
5	Fill a syringe with approximately 10 ml of the storage solution. Connect the syringe to the pH valve port **Cal**, and inject the storage solution. 
6	Press Close. *Result:* The pH valve switches back to the default position and the ***Calibration*** dialog closes. No calibration is performed.

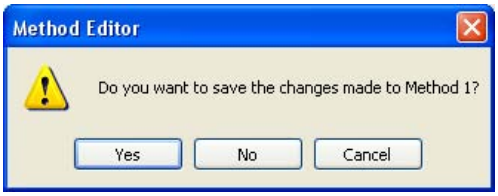
Log off or exit UNICORN

Follow the instruction below to log off or exit UNICORN. This can be performed from any of the UNICORN modules.

If you want to...	then...
log off UNICORN	select ***File:Log off***.  *Result*: All open UNICORN modules close and the ***Log On*** dialog opens.

If you want to...	then...
exit UNICORN	select ***File:Exit UNICORN***.  *Result*: All open UNICORN modules close.

Note: *If an edited method or result is open and not saved when you try to exit or log off UNICORN, you will see a warning. Click **Yes** to save, **No** to exit without saving, or **Cancel** to stay logged on.*



Shut down the instrument

Switch off the instrument by pressing the **Power** switch to the **O** position.

9 Evaluate and print the results

Introduction

This chapter describes how to use the ***Evaluation*** module to evaluate and print the results of a run. For further information, please refer to *UNICORN 6 Evaluation Manual*.

Contents

This chapter contains the following sections:

9.1 View the results

Introduction

A result holds a complete record of the run, including method, system settings, chromatogram, and run log. This section describes how to view a result and how to change the view settings in the ***Evaluation*** module.

Open a result

Follow the instruction below to open a result.

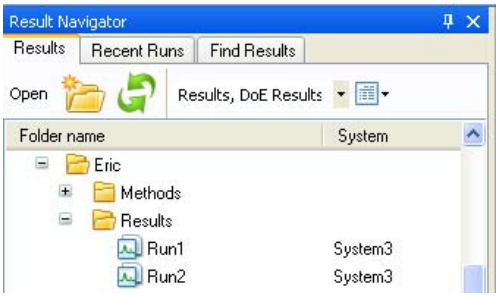
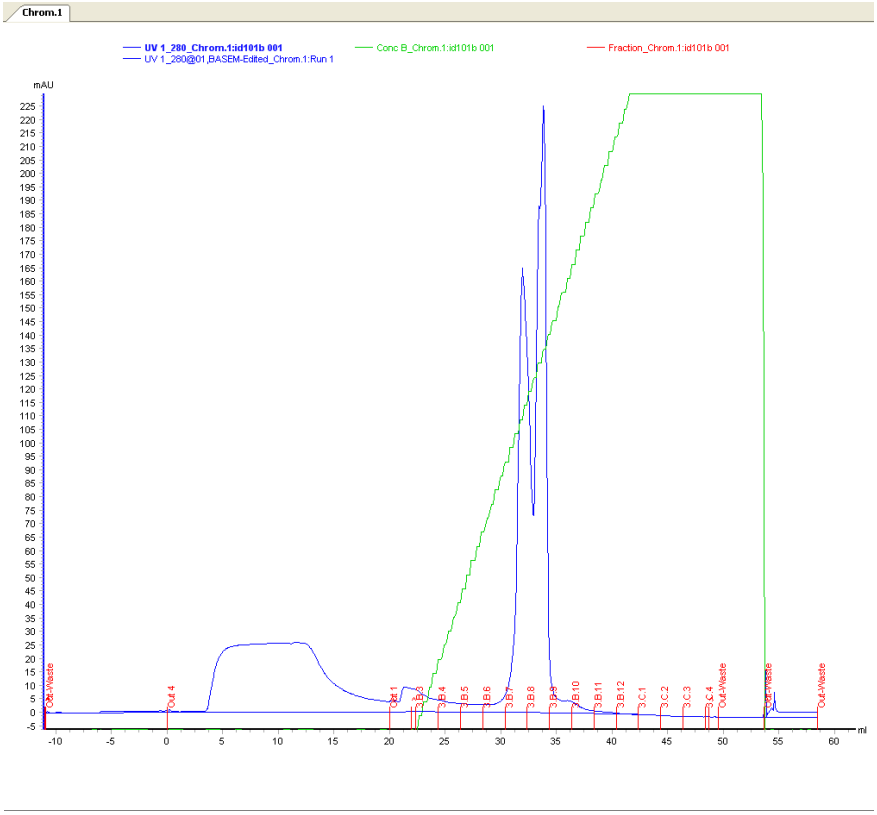
Step	Action
1	Open the ***Evaluation*** module and click the ***Open Result Navigator*** icon.  *Result*: The ***Result Navigator*** opens. 
2	Select the ***Results*** tab.
3	Select the result to open and click the ***Open a Result*** button in the navigator toolbar.  *Result*: The result is opened in a ***Chromatogram*** pane. Available chromatograms and peak tables are displayed.

Illustration of the Chromatogram pane

The ***Chromatogram*** pane displays the curves from the method run.



Customize Chromatogram

To change the chromatogram properties:

- Click the ***Customize*** icon to open the ***Customize*** dialog.

- Make appropriate selections under the tabs described in the table below.

Tab	Customization
Curves	Select curves to be displayed.
Curve style and color	Customize the appearance of the displayed curves.
X-axis	Select base unit (time, CV or volume) and set X-axis scale.
Y-axis	Select which y-axes to display and set Y-axis scale for the different curves.
Header	Select which parameters (variables, questions and/or notes) to display in the header information at top of the chromatogram.

- Click ***OK*** to save the changes and close the dialog.

Note: *These operations determine which curves are displayed and their appearance in the* ***Chromatogram*** *pane and on printouts. The original raw data curves can never be modified or removed from the result.*

View details in Chromatogram

Follow the instructions below to view selected parts of the chromatogram.

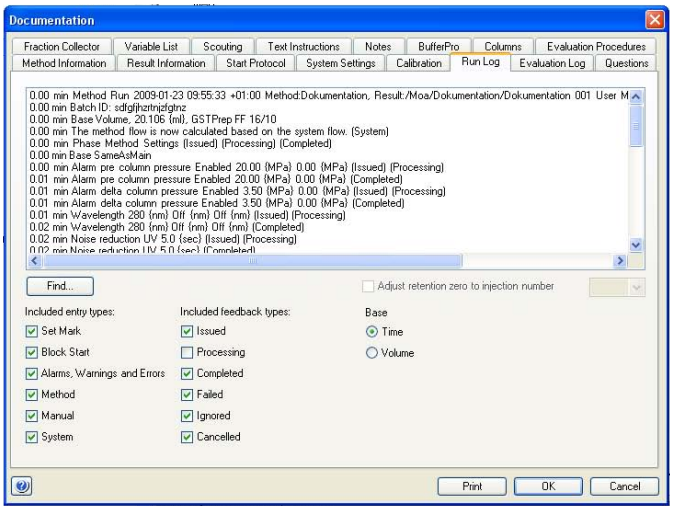
If you want to...	then...
identify a curve	position the mouse pointer over the curve of interest. *Result*: The curve name is displayed.
select which curve the Y-axis scale refers to	click the curve of interest. *Result*: The Y-axis scale changes to the unit applicable for the curve.
zoom in on the curves	select the area of interest by moving the pointer with the left mouse button held down. *Result*: The selected area is enlarged.
zoom out again	click the right mouse button and select ***Reset Zoom***. *Result*: The original area is restored in one step.

View documentation

To view documentation of the run:

- Click the ***View Documentation*** icon to open the ***Documentation*** dialog.

- Select the tab of interest. Which tabs are displayed depends on the settings in the active method.
- Click ***OK*** to close the dialog.



9.2 Peak integration

Introduction

Peak integration is used to identify and measure curve characteristics, including peak areas, retention times and peak widths. This section describes how to perform peak integration using the UNICORN software. For further information on peak integration, please refer to *UNICORN 6 Evaluation Manual*.

Perform peak integration

Follow the instruction below to integrate the curves in a result.

Step	Action
1	Open the ***Evaluation*** module and open a result from the ***Result Navigator***.
2	Click the ***Peak Integrate*** icon.  *Result*: The ***Peak Integrate*** dialog opens.

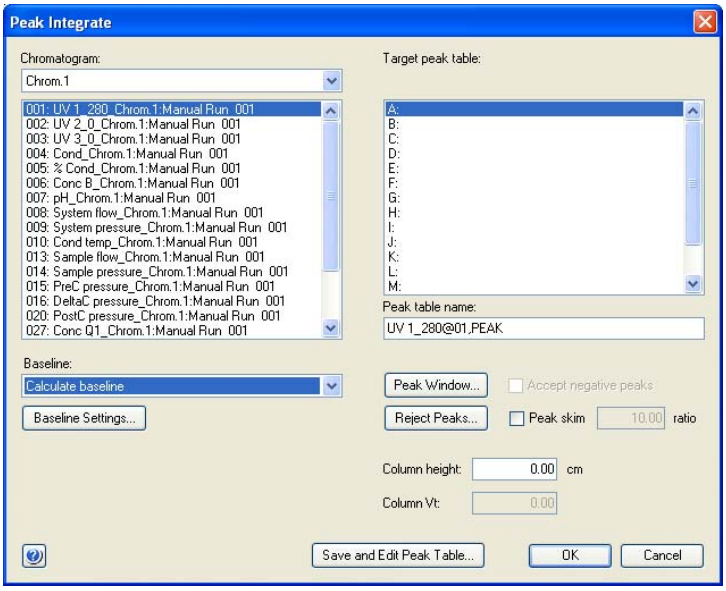
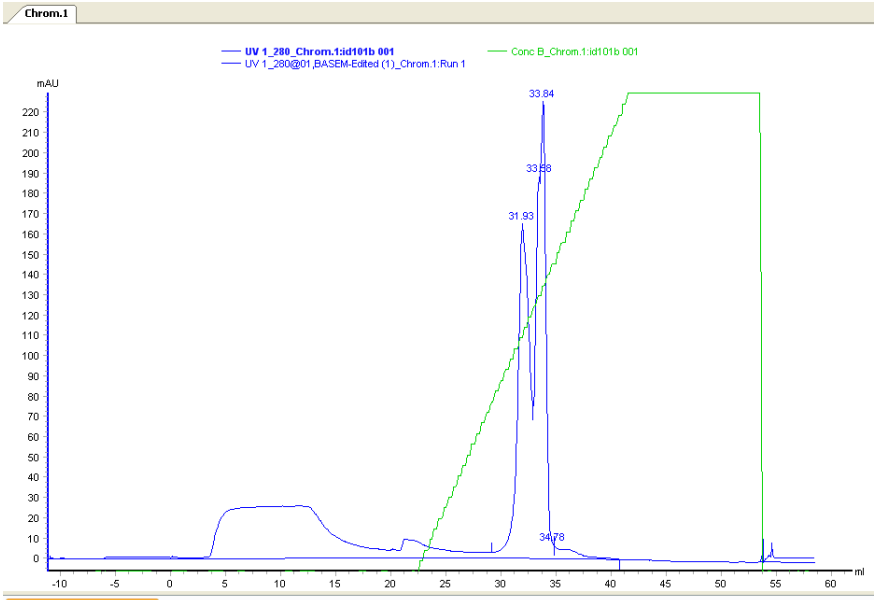
Step	Action
3	In the ***Peak Integrate*** dialog: • Select which curve to integrate (e.g., ***UV1_280*** for proteins). • Select a target peak table for storage of the result. • Select ***Calculate baseline*** from the ***Baseline*** list. • Click ***OK***.  *Result*: The ***Peak Table*** is displayed beneath the active chromatogram, and the start and end points of each peak are marked by vertical marks in the chromatogram.

Illustration of Chromatogram and Peak Table

The illustration below shows a ***Chromatogram*** pane including a ***Peak Table***.



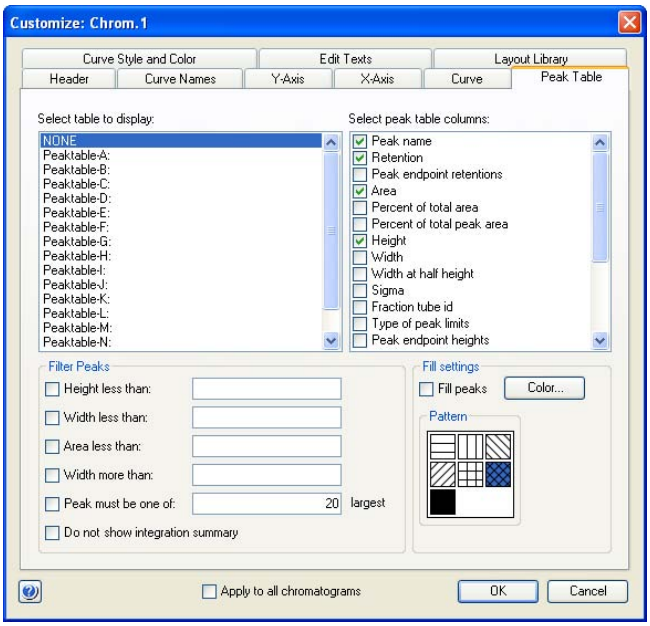
No	Peak name	Retention (ml)	Area (ml*mAU)	Height (mAU)
1		31.93	206.2683	164.965
2		33.58	94.1728	188.497
3		33.84	121.6052	225.321
4		34.78	13.1725	6.747

Customize Peak Table

By default, each peak in the ***Peak Table*** is displayed with its retention time, area and height. Follow the instruction below to display other peak data.

- Click the ***Customize*** icon to open the ***Customize*** dialog.

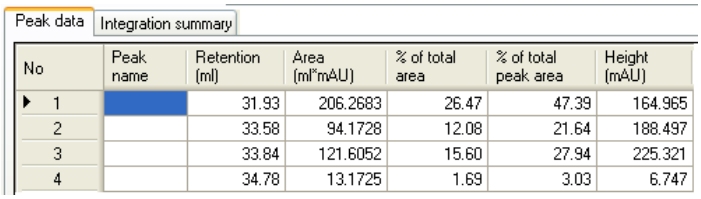
- Under the ***Peak Table*** tab:
  - Select which data to display in the peak tables from the ***Select peak table columns*** list.
  - Click ***OK*** to display the settings and close the dialog.



Study peak characteristics

The peaks in the integrated chromatogram are automatically labeled with their retention values. The ***Peak Table*** is calculated using the same base unit (ml, CV or min) as selected in the chromatogram. Follow the instruction below to find the peak of interest.

Step	Action
1	Find the retention value above a peak in the ***Chromatogram***.
2	Search the ***Peak Table*** for the same retention value.
3	On the same row, find the property of interest (e.g., ***Area***).



Peak data | Integration summary

No	Peak name	Retention (ml)	Area (ml*mAU)	% of total area	% of total peak area	Height (mAU)
1		31.93	206.2683	26.47	47.39	164.965
2		33.58	94.1728	12.08	21.64	188.497
3		33.84	121.6052	15.60	27.94	225.321
4		34.78	13.1725	1.69	3.03	6.747

Save results

Follow the instruction below to save your changes.

If you want to...	then...
save your changes to the original result	click the ***Save*** icon in the toolbar. 
save the changed result as a new result	• select ***File:Save As*** to open the ***Save Result As*** dialog. • In the ***Save Result As*** dialog: - select the location for the new result - write a name for the new result - click ***Save***.

9.3 Print the results

Introduction

This section describes how to print a chromatogram and a standard format report. UNICORN uses the printers and printer settings that are installed on your computer.

Customize the view

Before printing, make sure that the view is adjusted to show what is needed in the report/printout. Customize the chromatogram and/or peak table and view details in the chromatogram according to the instructions above. Before printing, check that:

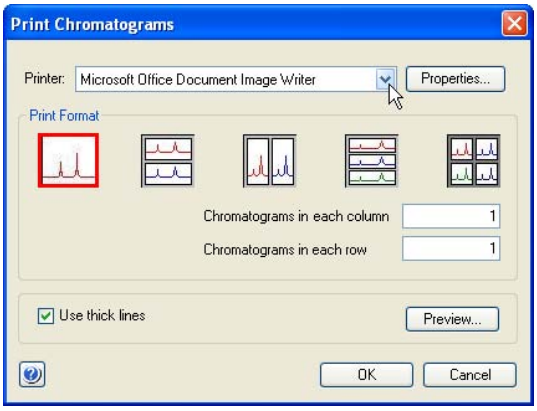
- correct curves are displayed
- correct data are displayed in the peak table
- a suitable scaling of the axes has been selected
- a suitable zoom factor has been selected

Print a chromatogram and a peak table

Follow the instruction below to print a chromatogram and, if peak integration has been performed, the related peak table.

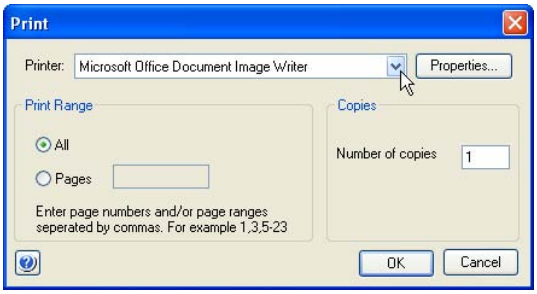
Step	Action
1	Open the ***Evaluation*** module and open a result.
2	Click the ***Print*** icon.

Result: The ***Print Chromatograms*** dialog opens.



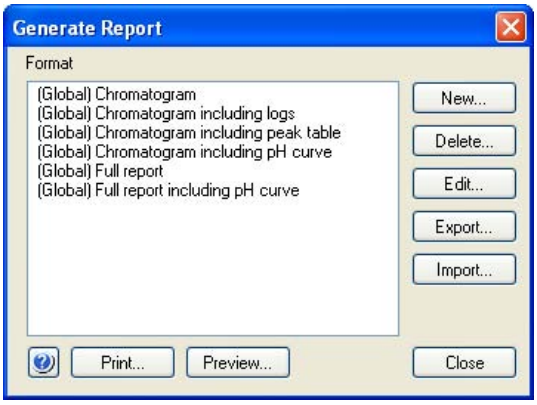
3	In the ***Print Chromatograms*** dialog: • Select ***Printer*** and ***Print Format***. • Click the ***Preview*** button.

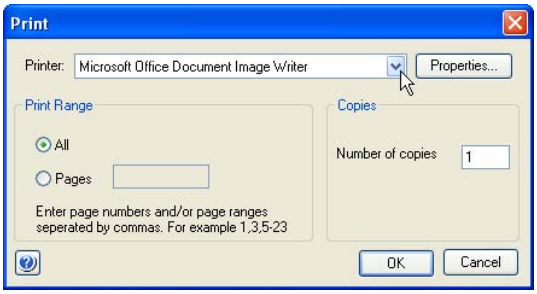
Result: A preview of the chromatogram displays in the ***Customize Report*** window.

Step	Action
4	Are you satisfied with the layout? • If Yes, continue to step 5. • If No, select ***File:Exit*** to return to the ***Print Chromatograms*** dialog and choose another print format.
5	In the ***Customize Report*** window, click the ***Print*** icon.  *Result:* The ***Print*** dialog opens. 
6	In the ***Print*** dialog, select ***Printer*** from the list and click ***OK***. *Result:* Your chromatogram is printed.

Print a report

Follow the instruction below to print a standard format report.

Step	Action
1	Open the ***Evaluation*** module and open a result.
2	Click the ***Report*** icon.  *Result*: The ***Generate Report*** dialog opens. 
3	In the ***Generate Report*** dialog: • Select any of the predefined formats from the list. • Click the ***Preview*** button.  *Result*: A preview of the report displays in the ***Customize Report*** window.
4	Are you satisfied with the layout? • If Yes, continue to step 5. • If No, select ***File:Exit*** to return to the ***Generate Report*** dialog and choose another format.

Step	Action
5	In the ***Customize Report*** dialog, click the ***Print*** icon.  *Result:* The ***Print*** dialog opens. 
6	In the ***Print*** dialog, select ***Printer*** from the list and click ***OK***. *Result:* Your report is printed.

10 Maintenance

Introduction

This chapter provides schedules for preventive maintenance that should be performed by the user of ÄKTAavant. Regular maintenance is essential for reliable function and results. Refer to ÄKTAavant and UNICORN 6 User Manual for detailed instructions.

WARNING

Always use appropriate personal protective equipment during operation and maintenance of ÄKTAavant system.

Contents

This chapter contains the following sections:

10.1 Maintenance program

Introduction

This section describes the preventive maintenance to be performed on ÄKTAavant.

The maintenance is divided into:

- Daily maintenance
- Weekly maintenance
- Monthly maintenance
- Bi-annual maintenance
- Maintenance when required

Periodic maintenance program

The following periodic maintenance should be performed by the user of ÄKTAavant.

Interval	Maintenance action
Daily	Calibrate the pH monitor
Weekly	Calibrate pressure monitors
Weekly	Change pump rinsing solution
Monthly	Check the flow restrictor
Bi-annual	Clean the UV flow cell
Bi-annual	Replace pH electrode

Maintenance when required

The following maintenance should be performed by the user of ÄKTAavant when required.

Maintenance action
Clean the instrument externally
Perform System CIP
Perform Column CIP
Clean the Fraction collector
Replace tubing and connectors
Storage of pH electrode
Clean the pH electrode
Clean the conductivity flow cell
Calibrate the Conductivity monitor
Calibrate the UV monitor
Replace mixer
Replace online filter
Replace o-ring in mixer
Replace UV flow cell
Replace flow restrictor
Replace inlet filters
Clean the check valves
Replace check valves
Replace pump piston seals
Replace pump pistons
Replace pump rinsing system tubing
Replace valve modules

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